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Juliette Chassain

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Biodiversité des sols et services écosystémiques associés dans des systèmes de culture alternatifs stables et en transition

*Soil biodiversity and associated ecosystem services in stable and
transitional alternative cropping systems*

Thèse de doctorat de l'université Paris-Saclay

École doctorale n°581 : agriculture, alimentation, biologie, environnement, santé (ABIES)
Spécialité de doctorat : Ecologie
Graduate School : Biosphera. Référent : AgroParisTech

Thèse préparée dans l'UMR **ECOSYS** (Université Paris-Saclay, INRAE, AgroParisTech),
sous la direction de **Laure VIEUBLE-GONOD**, Maîtresse de Conférences (HDR)
et le co-encadrement de **Sophie JOIMEL**, Maîtresse de Conférences

Thèse soutenue à Paris-Saclay, le 11 avril 2023, par

Juliette CHASSAIN

Composition du Jury

Membres du jury avec voix délibérative

Mickaël HEDDE Directeur de Recherche, INRAE (centre Occitanie-Montpellier)	Président & Rapporteur
Séverine PIUTTI Professeure, Université de Lorraine	Rapporteuse & Examinatrice
Céline PERNIN Maîtresse de Conférences, Université de Lille	Examinatrice
Jean-François VIAN Enseignant-Chercheur, ISARA Lyon	Examineur

Titre : Biodiversité des sols et services écosystémiques associés dans des systèmes de culture alternatifs stables et en transition

Mots clés : agriculture biologique, agriculture de conservation, faune du sol, communautés microbiennes, traits fonctionnels, fonctionnement du sol

Résumé : Les organismes du sol jouent un rôle clé dans le fonctionnement des agroécosystèmes. Or, l'agriculture intensive exerce de nombreuses perturbations qui affectent la biodiversité des sols. Le développement de systèmes alternatifs basés sur de moindres perturbations physiques et chimiques du sol, tels que l'agriculture biologique et l'agriculture de conservation, pourrait permettre de minimiser l'impact de l'agriculture sur les organismes du sol et d'améliorer les services écosystémiques associés. Toutefois, l'effet de ces systèmes sur les organismes du sol est encore mal connu, que ce soit à long terme ou lors de la période de transition. Dans ce contexte, l'objectif de cette thèse est de caractériser l'effet des systèmes de culture alternatifs stables et en transition sur la biodiversité du sol et les services écosystémiques associés en comparaison à l'agriculture conventionnelle en grande culture. Afin de répondre à cet objectif, des prélèvements ont été réalisés sur 21 parcelles chez des agriculteurs durant deux automnes consécutifs (2020 et 2021), afin d'étudier la densité et la diversité taxonomique et fonctionnelle des organismes du sol (mésos- et macrofaune, microorganismes) et leurs interactions, et de mesurer un ensemble de fonctions associées à leur activité dans le sol (décomposition de la litière, minéralisation du C et N, stabilisation de la structure). Les systèmes de cultures étudiés ont été caractérisés selon l'intensité des pratiques (labour, traitements phytosanitaires, fertilisation). Nos résultats montrent un effet positif des systèmes alternatifs sur la biodiversité du sol, mais qui varie selon les années et les groupes d'organismes. L'agriculture de conservation a un effet positif sur la densité de plusieurs groupes de la faune du sol tandis que l'agriculture biologique a

peu d'effets significatifs mais tend à favoriser la densité des microorganismes. Des effets transitoires sont observés au sein des parcelles récemment converties vers ces systèmes alternatifs, tels qu'une augmentation ou une diminution de la densité de certains groupes d'organismes et des différences dans la structure des communautés par rapport au système d'origine et aux systèmes alternatifs stables. La prise en compte de l'intensité des pratiques permet de mieux caractériser l'effet des systèmes sur la biodiversité du sol. L'intensité du labour est le principal facteur d'influence de la densité, la diversité et des traits fonctionnels des organismes. Une diminution de l'intensité du labour favorise la densité et la diversité de la faune du sol et les interactions entre organismes au sein du réseau trophique du sol. D'autre part, une revue de la littérature (i.e. méta-analyse) ainsi que les mesures *in situ* à l'aide de sachets de litière ont mis en évidence le rôle majeur de la méso- et de la macrofaune du sol dans la décomposition de la litière en milieu agricole. Toutefois, la décomposition de la litière et les autres fonctions étudiées dans cette thèse sont influencées par les systèmes de culture et l'intensité des pratiques. L'agriculture de conservation et une faible intensité du labour favorisent la formation d'agrégats stables. L'agriculture biologique ne présente pas d'effet significatif mais une faible intensité des traitements phytosanitaires favorise la décomposition de la litière tandis qu'une augmentation des apports de matière organique est liée à une minéralisation plus lente. Ainsi, ce travail démontre l'importance de la prise en compte de la biodiversité du sol pour le développement de systèmes de culture durables.

Title : Soil biodiversity and associated ecosystem services in stable and transitional alternative cropping systems

Keywords : organic farming, conservation agriculture, soil fauna, microbial communities, functional traits, soil functioning

Abstract : Soil organisms play a key role in the functioning of agroecosystems. However, intensive agriculture causes a wide range of disturbances affecting soil biodiversity. The development of alternative systems relying on lower physical and chemical disturbances of soil, such as organic and conservation agriculture, could limit the impact of agriculture on soil organisms and improve associated ecosystem services. Yet, the effect of these systems on soil organisms remains poorly understood, both at long-term and during the transition period. In this context, the objective of this thesis is to characterize the effect of stable and transitional alternative systems on soil organisms and associated ecosystem services compared to conventional agriculture for field crops. This was addressed through samplings in 21 fields owned by farmers during two consecutive autumns (2020 and 2021) in order to study the density and taxonomic and functional diversity of soil organisms (meso- and macrofauna, microorganisms) and their interactions, and to measure soil functions associated to their activity in soil (litter decomposition, C and N mineralization, stabilization of the soil structure). The studied cropping systems were characterized by assessing practice intensity (tillage, pesticide treatments, fertilization). The obtained results show an overall positive effect of alternative systems on soil biodiversity, but this effect varied with years and taxa. Conservation agriculture has a positive effect on the density of several soil fauna groups,

whereas organic agriculture has few significant effects but tends to promote microbial density. Transitory effects are observed within fields recently converted to these alternatives, such as an increase or a decrease in the density of some groups of organisms or differences in the community structure compared to initial and to stable alternative systems. Practice intensity provides more insight on the effect of cropping systems on soil biodiversity. Tillage is the main factor influencing the density, diversity and functional traits of soil organisms. A decrease in tillage intensity promotes the density and diversity of soil fauna and the interactions between soil organisms within the soil food web. Additionally, a literature review (i.e. meta-analysis) as well as *in situ* measurements of litter decomposition with litterbags demonstrated the main role of soil meso- and macrofauna in litter decomposition in agricultural soils. However, litter decomposition and other studied functions are influenced by cropping systems and practice intensity. Conservation agriculture and a low tillage intensity promote stable soil aggregates. Organic agriculture does not show significant effects, but a low pesticide treatment intensity may favor litter decomposition, and more organic matter inputs are related to a slower C and N mineralization. Thus, this work demonstrates the importance of taking into account soil biodiversity for the development of sustainable cropping systems.

Avant-propos

Cette thèse a été menée au sein de l'équipe Sol (devenue Sol&Tox) qui fait partie de l'UMR ECOSYS (Université Paris-Saclay, INRAE, AgroParisTech) située jusqu'à récemment sur le site de Thiverval-Grignon et délocalisée sur le plateau de Saclay. Elle a été dirigée par Laure Vieublé Gonod (AgroParisTech) et co-encadrée par Sophie Joimel (AgroParisTech). Ces travaux ont été financés par une bourse obtenue auprès de l'école doctorale ABIES, et leur fonctionnement s'est appuyé sur les projets Sys&Div (« Combiner des systèmes de culture et des aménagements parcellaires reposant sur la biodiversité pour augmenter la multifonctionnalité de l'agriculture ») et DYNABIO (« Dynamique de la biodiversité des sols et des services associés dans des systèmes de culture en transition ») soutenus par l'INRAE, AgroParisTech et l'Office français de la biodiversité.

Le manuscrit comprend une introduction générale, six chapitres de résultats rédigés sous forme d'articles scientifiques ainsi qu'une discussion et conclusion générale. Les références bibliographiques sont regroupées à la fin du manuscrit. Le format du manuscrit induit inévitablement des redondances, en particulier entre l'introduction et les parties matériels et méthodes des différents chapitres.

Le chapitre 5 a été publié dans la revue *Soil Biology and Biochemistry* (Chassain et al., 2021). Les autres chapitres, déjà rédigés au format article, seront valorisés ultérieurement.

L'ensemble du manuscrit est rédigé en anglais, mis à part cet avant-propos et les remerciements, afin de permettre une diffusion à l'international et une accessibilité au plus grand nombre de l'ensemble des travaux réalisés. Un résumé substantiel de la thèse en français est disponible en fin de manuscrit.

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———— General Introduction ————

I.1 Context

Soil is the habitat of an unsuspected biodiversity representing up to 25% of described species in terrestrial ecosystems (Decaëns et al., 2006). Yet, a large part of this biodiversity remains unknown and soil is considered as one of the last biotic frontiers, together with ocean abysses and tree canopies (André et al., 1994). Furthermore, it is widely recognized that soil organisms perform essential soil functions, thus contributing to the provision of many ecosystem services (Lavelle et al., 2006; Brussaard et al., 2007; Kibblewhite et al., 2008).

However, terrestrial ecosystems are subjected to a broad range of anthropic disturbances that are responsible for environmental degradation and an important loss of biodiversity. Among the various activities responsible for these disturbances, intensive crop production is probably the one that affects the largest area on Earth (Robertson and Swinton, 2005). The intensification of agriculture since the green revolution successfully increased crop yield thanks to synthetic inputs and mechanization but caused considerable damage to the environment (Pimentel and Pimentel, 1990) and to biodiversity (Tsiafouli et al., 2015; Beckmann et al., 2019). This conducts to a decrease in ecosystem services such as pest regulation and water and erosion control, and to the formation of a vicious circle where more inputs are added to prevent yield loss (Pimentel and Pimentel, 1990). Thus, intensive agriculture is not adapted to sustainably meet the challenges caused by a growing population. In addition, climate change hastens the need for the development of systems able to mitigate or adapt to the forthcoming changes.

In 2019, the IPBES designated agriculture as a main threat to biodiversity and ecosystems and stated “feeding humanity and enhancing the conservation and sustainable use of nature are complementary and closely interdependent goals that can be advanced through sustainable agriculture [...]. Specific actions include promoting sustainable agricultural and agroecological practices” (Watson et al., 2019). Currently, the main alternatives to intensive agriculture are organic and conservation agriculture, based on lower chemical and physical disturbances respectively. These alternatives however present some drawbacks, namely strong tillage intensity in organic systems and a large use of herbicides in conservation systems. New alternatives could thus be developed in the following years, accounting for instance for the ban of glyphosate or the reduction of mechanical operations and production of synthetic fertilizers, all of which rely on fossil fuel. The development of sustainable systems requires understanding the consequences of cropping systems and practices on the different components of ecosystems, including all compartments of the biodiversity. However, in agricultural areas most studies were conducted on aboveground biodiversity. The effect of alternative cropping systems and practices on soil biodiversity remains largely unknown and there is no clear consensus on the way to promote soil

organisms. Therefore, there is an urgent need for more knowledge on soil biodiversity and on associated functions under different alternative systems.

In this context, this PhD thesis aims to compare the effects of alternative cropping systems, stable or in transition, and conventional systems on soil organisms and associated ecosystem services in annually cropped soils. This manuscript starts with a general introduction followed by six chapters of results and ends with a general discussion and conclusion. The general introduction summarizes the current state of knowledge on the effects of cropping systems on soil biodiversity, and presents the existing methods for describing cropping systems and soil biodiversity, as well as the study area and the experimental approach of the thesis. The first three chapters of results present the effect of cropping systems and practice intensity on soil mesofauna, macrofauna and microorganisms. The fourth chapter aims to synthesize the effect of cropping systems on soil biodiversity and to assess the effects of practice intensity on interactions within the soil food web. Finally, the fifth and sixth chapters assess soil functions driven by soil organisms in an agricultural context.

I.2 State of the art

I.2.1 Current challenges on agricultural soils

Soils are an essential component of terrestrial ecosystems and play a crucial role in food security, climate regulation and biodiversity conservation through the provision of many ecosystem services. However, they are subjected to anthropic disturbances causing their degradation with erosion, loss of organic matter and loss of biodiversity (Bridges and Oldeman, 1999). Among them, agricultural practices represent one of the main anthropic disturbances on terrestrial ecosystems.

Agricultural soils are the support of crop production but they can also provide many other services such as water quality and supply, erosion control and climate regulation (Robertson and Swinton, 2005; Kibblewhite et al., 2008). However, soils in agricultural areas are under stress as they are regularly subjected to a wide range of physical and chemical disturbances caused by crop management practices (e.g. tillage, pesticides). In intensive systems, deep tillage, pesticide inputs and exportation of organic matter reportedly conduct to changes in the soil structure and to the impoverishment of soils on the long-term (Reeves, 1997; Or et al., 2021).

Nowadays, agriculture is at a turning point where it needs to develop alternative systems relying on lower physical and chemical disturbances of soil in order to prevent further degradations, while maintaining acceptable food production. Yet, we still lack knowledge on the effect of cropping systems on soils and on the best practices to promote higher soil quality. Indeed, soils, and particularly soil biodiversity, are largely understudied due to a lack of interest and to a misconception of their importance in the provision of ecosystem services for a long time. The 4 per 1000 initiative launched at the COP21 in 2015 was a first step to raise awareness on the potential of agricultural soils to store carbon and limit climate change, and to attract attention on the impact of agricultural practices on soils (Chenu et al., 2019). In 2020, the European Joint Programme Cofund on Agricultural Soil Management (EJP Soil) went further with the objectives to generate knowledge, tools and a research community that will promote climate-smart sustainable agricultural soil management to sustain food production, biodiversity and soil functions (ejpsoil.eu). More generally, current research aims to monitor and to develop agricultural practices ensuring a good soil quality (see Box 1) from which soil biodiversity is a crucial component (Lavelle et al., 2006; Kibblewhite et al., 2008; Bünemann et al., 2018). In order to provide relevant advices on practices that promote a good soil quality, it is hence necessary to understand cropping system effects on soil biodiversity and the related soil functions.

Box 1. Definition of soil quality

Soil quality can be defined as “the capacity of a soil to function as a vital living system, within ecosystem and land use boundaries, to sustain plant and animal productivity and health, maintain or enhance water and air quality, and to further provide ecosystem services on the long-term without increased tradeoffs between ecosystem services” (EJP Soil, after Doran et al., 1996; Karlen et al., 1997; Giuffré et al., 2021). Soil quality encompasses all levels from soil structures to ecosystem services, including soil processes and functions (Figure I.1). The promotion of soil quality is included in several of the United Nations Sustainable Development Goals (SDG) and in the European Green Deal.

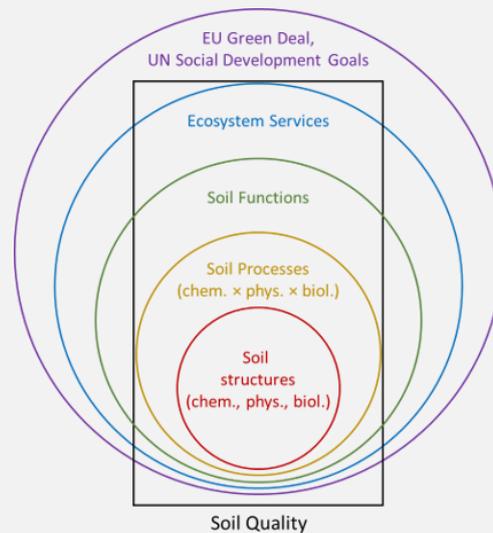


Figure I.1 Concepts related to soil quality as defined by the SIREN project of the EJP soil (ejpsoil.eu)

I.2.2 Soil biodiversity in agroecosystems

Agricultural soils host a wide range of soil organisms within soil porosity, around plant roots and on the surface layer formed by crop residues. Some of the main representatives of this underground life are earthworms, Collembola, fungi and bacteria, but soil communities are among the most diverse within terrestrial ecosystems (Lavelle et al., 2006) and knowledge on soil taxa is still scarce compared to aboveground taxa (Decaëns, 2010). Soil organisms are usually grouped according to their size (Swift et al., 1979; Lavelle and Spain, 2001) (Figure I.2). Soil microorganisms (i.e. microflora) and microfauna include organisms of less than 0.1 mm (e.g. bacteria, fungi, nematods), soil mesofauna ranges from 0.1 to 2 mm (e.g. Collembola, Acari) and soil macrofauna is larger than 2 mm (e.g. earthworms, ground beetle). Megafauna comprises animals larger than invertebrates. This classification aims at distinguishing the level of action of organisms (micro to macro scale) and the means of extraction and identification of the organisms.

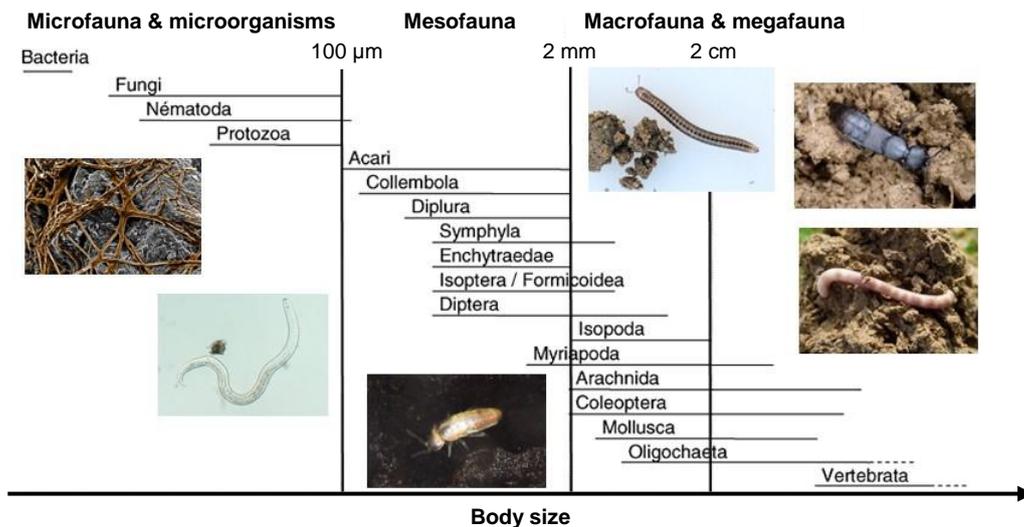


Figure 1.2 Classification of the main groups of soil organisms by size (adapted from Decaëns et al., 2010; after Swift et al., 1979).

Soil organisms play a key role in the functioning of agrosystems (Lavelle et al., 2006; Brussaard et al., 2007; Chen et al., 2020). They are involved in a wide range of soil functions and processes that foster soil fertility, structural stability and carbon storage. Each function relies on the activities performed by diverse soil organisms, on their interactions and on soil properties (Kibblewhite et al., 2008). Among other functions, soil fauna drives litter decomposition (Swift et al., 1979; Frouz et al., 2015; Njoroge et al., 2022). Macrofauna detritivores and earthworms are involved in litter decomposition through direct action of breakdown, digestion and mixing of litter in soil (Barrios, 2007; Frouz, 2018). Microarthropods enhance decomposition partly through litter comminution, but mostly through grazing on microorganisms that stimulates microbial growth and indirectly increases decomposition (Seastedt, 1984; Culliney, 2013). Microorganisms control soil carbon (C) and nitrogen (N) mineralization, which can be indirectly promoted by the action of earthworms on soil (Hedde et al., 2013; Bertrand et al., 2015). The formation and stabilization of soil aggregates are fostered by the synthesis of molecules (e.g. glomalin, enzymes) by bacteria and fungi (Lehmann and Rillig, 2015), and supported by soil fauna such as earthworms and Collembola (Six et al., 2002; Maaß et al., 2015). Overall, soil organisms indirectly or directly promote plant growth (Kulmatiski et al., 2014; Majeed et al., 2018; Ramakrishna et al., 2019) and take part in pest regulation (Kromp, 1999; Lavelle et al., 2004), water circulation (Neher, 1999) and ecosystem resistance and resilience to stress (Brussaard et al., 2007; van Bruggen, 2014).

The soil system is complex as soil functioning is driven by soil organisms that have complementary roles and can act at different spatio-temporal scales, in particular on soil organic matter degradation and soil structural stability. Thus, soil quality should be assessed through the measurement of multiple soil organisms and functions (Kibblewhite et al., 2008). However, few studies simultaneously assess several groups of soil organisms and their interactions due to limitation in time, identification knowledge and costs.

I.2.3 Effects of intensive cropping systems and practices on soil organisms

Despite their important role in agricultural soils, soil organisms have a lower density and diversity in cropping systems than in any other ecosystems (Spurgeon et al., 2013; Santorufu et al., 2015; Joimel et al., 2017). Indeed, in order to increase crop yield, intensive systems have progressively replaced the role performed by soil organisms by the use of synthetic inputs and fossil-fuel based mechanization (Giller et al., 1997; Kibblewhite et al., 2008). Intensive agriculture nowadays relies on deep tillage, large quantities of pesticide products and mineral fertilizers, and short crop rotations (see Box 2).

Intensive systems are reported to negatively affect all the groups of soil organisms (Ponge et al., 2013; Tsiafouli et al., 2015). Previous studies notably reported negative effects on the density and diversity of microorganisms (Ponge et al., 2013; Karimi et al., 2018), microarthropods (Rusek, 1998; Bedano et al., 2006), earthworms (Tsiafouli et al., 2015; Briones and Schmidt, 2017) and other macrofauna organisms (Eggleton et al., 2005; Yin et al., 2022). These results can be related to the impacts of intensive practices on the soil quality on the short- and long-term through modifications of soil physical, chemical and biological properties (Mazzoncini et al., 2010).

Agricultural practices are among the most important human activities on soils as they cause regular disturbances that shape the soil community and influence ecosystem processes (McLaughlin and Mineau, 1995; Bünemann et al., 2006). As a response to regular and important disturbances, a selection occurs within soil organisms. Therefore, agricultural soils are hosting organisms able to develop under changing conditions, with a short generation time, high reproduction and dispersal abilities, and a small body size compared to other ecosystem types (Kladivko, 2001; Tsiafouli et al., 2015). In line with this, the highest impact of intensive practices may occur on larger organisms (Postma-Blaauw et al., 2010, 2012) and on organisms of the higher trophic levels (Coudrain et al., 2016; Maisto et al., 2017). Research on agricultural soils mostly reported the effects of tillage, and in a lesser extent of crop protection treatments (i.e. pesticides) and fertilization.

Tillage is arguably the practice that affects soil communities the most. It is responsible for a redistribution of organic matter and affects numerous soil properties such as porosity and compaction, thus driving soil structure, water content and temperature (McLaughlin and Mineau, 1995; Pires et al., 2017). It notably decreases the abundance and continuity of macropores (Capowiez et al., 2009a). Disturbances related to tillage influence soil fauna density, diversity and community structure. The effects can be direct, namely physical injuries or entrapment in soil, or indirect through changes in the location of trophic resources (i.e. crop residues, weeds), destruction of habitats (i.e. burrows, soil pores, surface litter), desiccation of the soil surface and increased exposition to predation (e.g. birds) (Stubbs et al., 2004; Roger-Estrade et al., 2010;

Patterson et al., 2019). Despite a general negative effect of tillage reported on soil fauna density and diversity, the actual effect of tillage on soil organisms depends on the groups considered and on the soil conditions at the time of tillage (Kladivko, 2001). Larger organisms, notably earthworms, are more likely to undergo direct effects of tillage (Wardle, 1995; Chan, 2001; Kladivko, 2001). However, smaller fauna such as microarthropods can also be largely affected (van Capelle et al., 2012; Moradi et al., 2013), although effects may depend on soil properties and are not always observed (van Capelle et al., 2012; Rieff et al., 2020). In addition, tillage influences microbial communities both on the short- and long-term (Le Guillou et al., 2019; Kraut-Cohen et al., 2020; Romero-Salas et al., 2021). However, the effects of tillage on soil microorganisms are complex as tillage can have detrimental or positive effects on the microbial biomass and bacterial and fungal communities in association to environmental conditions (Beare et al., 1993; Stubbs et al., 2004; Anderson et al., 2017). Tillage fragments the network of fungal hyphae, hence decreasing fungal growth (Beare et al., 1993). Nevertheless, as it buries crop residues, weeds and organic amendments, tillage can also promote bacterial abundance and diversity (Le Guillou et al., 2019; Tyler, 2019; Olayemi et al., 2022) by increasing contact between microorganisms and trophic resources.

Pesticides influence on agroecosystems is largely studied, but most of the research is conducted on aboveground soil biodiversity. In soils, pesticide applications could be a second order factor of influence on organisms, as reported for microarthropods (Cortet et al., 2002b) and microorganisms (Hartmann et al., 2015). However, pesticides can affect untargeted soil species with potential feedbacks on the soil food web. Both soil fauna and soil microorganisms are especially sensitive to insecticides and fungicides (Beare et al., 1993; McLaughlin and Mineau, 1995; Bünemann et al., 2006). Detrimental effects of pesticides are observed in particular on fungi (Beare et al., 1993; Bünemann et al., 2006), Collembola (Cortet et al., 2002a; Miyazawa et al., 2002; Joimel et al., 2022) and earthworms (Pelosi et al., 2014; Datta et al., 2016). Conversely, inconsistent effects of pesticides were reported on soil microorganisms (Beare et al., 1993; Bünemann et al., 2006). This may be due to herbicide applications which lead to an increase in dead weed biomass, and hence in food resource for soil microorganisms (Neher, 1995). In addition, herbicides were reported to have transient effects on bacteria that disappear when molecules have been degraded, adsorbed or lixiviated (Vieublé Gonod et al., 2006).

Mineral fertilizers are used instead or in addition to organic matter inputs in order to promote faster assimilation of nutrients by crops. A meta-analysis revealed that mineral fertilizers have the potential to increase microbial biomass compared to unfertilized soils at long-term, but this effect depended on soil properties such as pH (Geisseler and Scow, 2014). In particular, the increase in plant biomass following mineral fertilization and additional crop residues return to soil can enhance the activity of soil organisms (Bünemann et al., 2006). However, long-term

application of mineral fertilizers can lead to a decrease in soil organic carbon and total nitrogen with consequences on the community composition of soil organisms (de Souza and Freitas, 2018). In addition, mineral fertilizers, contrary to organic matter inputs, cannot be used directly as a food source by soil fauna and part of the microorganisms. Thus, mineral fertilizers provide poor benefits to the vast majority of soil organisms and conduct to lower density and diversity of organisms compared to organic fertilizers (e.g. manure, compost). This was reported for microorganisms (Treonis et al., 2010; Rachwał et al., 2021), microarthropods (Miyazawa et al., 2002; Betancur-Corredor et al., 2023) and earthworms (Birkhofer et al., 2008; Capowiez et al., 2009b; Ponge et al., 2013). Contrasting results were however reported regarding soil fauna, for instance for microarthropods (Weil and Kroontje, 1979; Kanal, 2004; Coudrain et al., 2016). The type of applied fertilization can also strongly influence the community composition and abundance of soil organisms (de Souza and Freitas, 2018; Ren et al., 2019) with important changes reported within microbial communities (Schmid et al., 2018; Tosi et al., 2021). In addition, residues exportation cause a decrease in organic matter available at the surface, but this effect could be limited depending on fertilization inputs (Stumborg et al., 1996). Exports of residues were reported to have negative effects on the abundance and biomass of earthworms and to influence their community composition (Lemtiri et al., 2018; Frazão et al., 2019), but could benefit the abundance of Collembola (Brennan et al., 2006). In addition, residue exportations may decrease microbial biomass and cause changes in microbial communities (Govaerts et al., 2007).

Short crop rotation and monoculture can decrease the soil biodiversity (Patterson et al., 2019). Compared to longer rotation, short rotations may also lead to a lower abundance of microarthropods (Andrén and Lagerlöf, 1983; Jagers Op Akkerhuis et al., 2010) and lower microbial biomass and activity (Chahal et al., 2021; Singh and Kumar, 2021). Systems with short rotations are more subjected to the development of weeds and plant diseases and hence depend on higher pesticide inputs, which affect soil organisms (McLaughlin and Mineau, 1995).

Overall, intensive systems exert a wide of range of disturbances influencing the density and diversity of soil organisms, but there is no consensus on the effect of practices and on which practices are more disruptive than the others. In addition, the effect of combinations of practices on soil organisms are still poorly documented (Treonis et al., 2010).

I.2.4 Development of alternative cropping systems and effects on soil organisms

I.2.4.1 Effects of alternative cropping systems on soil organisms

Organic and conservation agriculture are currently considered as the main alternatives to conventional agriculture (see Box 2). They aim to reduce chemical and physical disturbances in order to promote soil functioning, and thus to rely on the associated ecosystem services. These alternative cropping systems were reported to increase the abundance and diversity of several taxonomic and functional groups of soil organisms compared to conventional agriculture (Henneron et al., 2015; Pelosi et al., 2015; Christel et al., 2021). In the last decades, a large part of the research on alternative cropping systems has been focused on organic agriculture. This is explained by the increasing use of organic farming following an increase in the demand for organic products, which is in relation to the negative image of pesticides. In contrast, few studies were conducted on conservation systems *stricto sensu*, especially in Europe. In this thesis, we discuss about previous results obtained under no-tillage and conservation systems in the same section, considering that they share a common primary principle, but they are two distinct systems.

Organic systems are based on the exclusion of synthetic inputs (pesticides, mineral fertilizers), therefore on low chemical disturbances, but strongly rely on mechanical weeding, which increases the level of physical perturbations (see Box 2). In their meta-analysis, Bengtsson et al. (2005) found that organic agriculture can lead to an increase of 30% of the total species richness compared to conventional systems. However, they also demonstrated a large variability across studies, with 16% of the studies reporting a negative effect of organic farming on species richness. Hole et al. (2005) additionally highlighted a high variability within studies and the need for further research to conclude on the effect of organic farming on biodiversity. Moreover, most studies were conducted on the aboveground biodiversity, which is less impacted by soil physical disturbances than the belowground biodiversity. In soils, organisms may benefit from organic systems due to lower inputs of pesticides, additional inputs of organic matter and associated increase in weed biomass, but they can also suffer from more regular and intense mechanical weeding and tillage. There is evidence that organic systems present an overall greater density and diversity of soil invertebrates than fields under conventional systems (Bengtsson et al., 2005; Hole et al., 2005; Henneron et al., 2015). Organic farming is notably reported to benefit the microbial biomass and the abundance and diversity of many soil fauna representatives, but contradictory results are also reported (Table I.1). Overall, the effect of organic agriculture vary between groups of soil organisms and opposite responses can be observed for density and diversity (Schrader et al., 2006; Patterson et al., 2019). More studies are thus required to assess the effects of organic systems on the belowground organisms.

Box 2. Definition of main cropping systems

Conventional agriculture, also called productive or intensive agriculture, is based on the maximization of yield and strongly relies on large inputs of synthetic fertilizers and pesticides. It also favors monoculture or short crop rotations including mostly high-yielding crops (i.e. wheat, maize) and varieties.

Organic agriculture consists in systems where synthetic fertilizers and pesticides are banned. It relies on organic matter inputs and on the use of legume crops for fertilization, and on high tillage intensity for weed regulation to compensate for the absence of pesticides. In addition, if synthetic products are banned from organic farming, some natural inputs such as plant preparation or biocontrol products are permitted. Organic agriculture is regulated by a European law since 1991 and relies on an official certification. Its aims are to insure crop production in the respect of environment, biodiversity, human health and animal well-being. In 2021, the surface of organic agriculture was estimated to reach 10.3% of the total arable surface in France (Agence Bio, Ministère de l'agriculture).

Conservation agriculture rests on three pillars, which are 1) minimum physical disturbances of soil with no-tillage and direct seedling, 2) permanent cover of soil with residues or cover crops, and 3) diversification of the crop rotation and potential crop associations (FAO, 2016). However, higher herbicides inputs are often used to manage weeds in the absence of tillage (Scopel et al., 2013; Colbach and Vacher, 2014). Conservation agriculture has first been employed in agricultural areas with the aims of controlling soil erosion and avoiding agricultural land degradation (Kassam et al., 2015), and then for promoting biological diversity, breaking the cycle of diseases, increasing carbon accumulation in the soil and limiting weeds (Santos et al., 2018). It does not rely on a strict certification as for organic farming and is less developed in Europe than in other parts of the world (e.g. North and South America) due to different stakes in the agricultural areas (Kassam et al., 2015). Conservation agriculture is regularly confused with no-tillage (also called conservation tillage), that is, the sole absence of tillage without necessarily involving the other aspects of conservation agriculture (Reicosky, 2015).

Conservation systems, on the other hand, are based on the combination of no-tillage, permanent soil cover (crop residues or mulch) and diversification of crop rotation, resulting in low physical disturbances of soil, but they tend to rely on higher herbicide inputs for weed management (see Box 2). Conservation agriculture could promote simultaneously provisioning (i.e. yield) and other ecosystems services, representing a good alternative to conventional systems (Chabert et al., 2020). However, compared to organic systems, there is a significant lack of studies assessing the effect of conservation systems on soil biodiversity (Christel et al., 2021). Under

conservation systems, soil organisms may benefit from the absence of mechanical disturbances and from a permanent soil cover (living cover crops, residues, mulch) providing habitat, food source and higher soil moisture (Wardle, 1995). Indeed, conservation systems are reported to increase the activity, density and diversity of soil organisms compared to conventional systems (Scopel et al., 2013; Henneron et al., 2015) (Table I.1). They can also foster soil microbial biomass in the first centimeters of soil but contrasting results are observed between studies. In addition, there is evidence for an increase in the abundance of several taxa of soil organisms, while few results are available on diversity (Table I.1).

Table I. 1 Synthesis of the effects of organic and conservation agriculture on the abundance, diversity and community structure of soil organisms based on some examples of the literature. Reported effects are either an increase (\nearrow), a decrease (\searrow) or neutral (\rightarrow).

	Organic agriculture	Conservation agriculture
Abundance & biomass	\nearrow : microbial biomass and activity (Birkhofer et al., 2008; Lori et al., 2017), microarthropods (Doles et al., 2001; Bettiol et al., 2002), macroarthropods (Maeder et al., 2002; Birkhofer et al., 2008), earthworms (Bettiol et al., 2002; Maeder et al., 2002; Pelosi et al., 2015)	\nearrow : microbial biomass (Sapkota et al., 2012; Palm et al., 2014), bacteria and fungi (Henneron et al., 2015), mesofauna (Ayuke et al., 2019; Dulaurent et al., 2022), macroarthropods (Ayuke et al., 2019), earthworms (Mele and Carter, 1999; Castellanos-Navarrete et al., 2012; Hernández et al., 2017; Dulaurent et al., 2022)
	\rightarrow or \searrow : microorganisms (Bettiol et al., 2002; Schrader et al., 2006), microarthropods (Schrader et al., 2006; Potapov et al., 2022), macroarthropods (Patterson et al., 2019), earthworms (Pelosi et al., 2009)	\rightarrow or \searrow : microbial biomass (Scopel et al., 2013)
Diversity	\nearrow : bacteria and fungi (Hartmann et al., 2015; Lupatini et al., 2017), microarthropods (Bettiol et al., 2002; Schrader et al., 2006), macroarthropods (Patterson et al., 2019), earthworms (Henneron et al., 2015)	\nearrow : mesofauna and macrofauna (Ayuke et al., 2019), fungi (Choudhary et al., 2018), bacteria (Singh et al., 2020)
	\rightarrow or \searrow : microarthropods (Alvarez et al., 2001; Doles et al., 2001; Filser et al., 2002; Peredo et al., 2009; Potapov et al., 2022), earthworms (Pelosi et al., 2009)	\rightarrow or \searrow : earthworms and Collembola (Dulaurent et al., 2022), fungi (Mhlanga et al., 2022; Zhang et al., 2022)
Community structure	Microorganisms (Hartmann et al., 2015), earthworms (Capowiez et al., 2009a), Collembola (Potapov et al., 2022)	Microorganisms (Romero-Salas et al., 2021; Zhang et al., 2022), earthworms (Pelosi et al., 2009)

Few studies assessed conjointly the role of different alternative systems. Henneron et al. (2015) reported a higher soil organisms abundance under organic and conservation systems compared to conventional systems, with a stronger overall enhancement for conservation than for organic systems (Figure I.3). However, the latter study was conducted in the experimental station of La Cage (Versailles, France) where organic plots are not amended with organic matter contrary to the huge majority of organic systems under real conditions. Therefore, further investigations are required to assess whether these differences between systems are observed under more realistic conditions. Furthermore, some other alternatives may be developed in the following years such as a combination between organic management and no-tillage. They will induce transitions whose consequences on biodiversity and soil functions are not well understood (Crittenden et al., 2014; Peigné et al., 2018; Rivers et al., 2020).

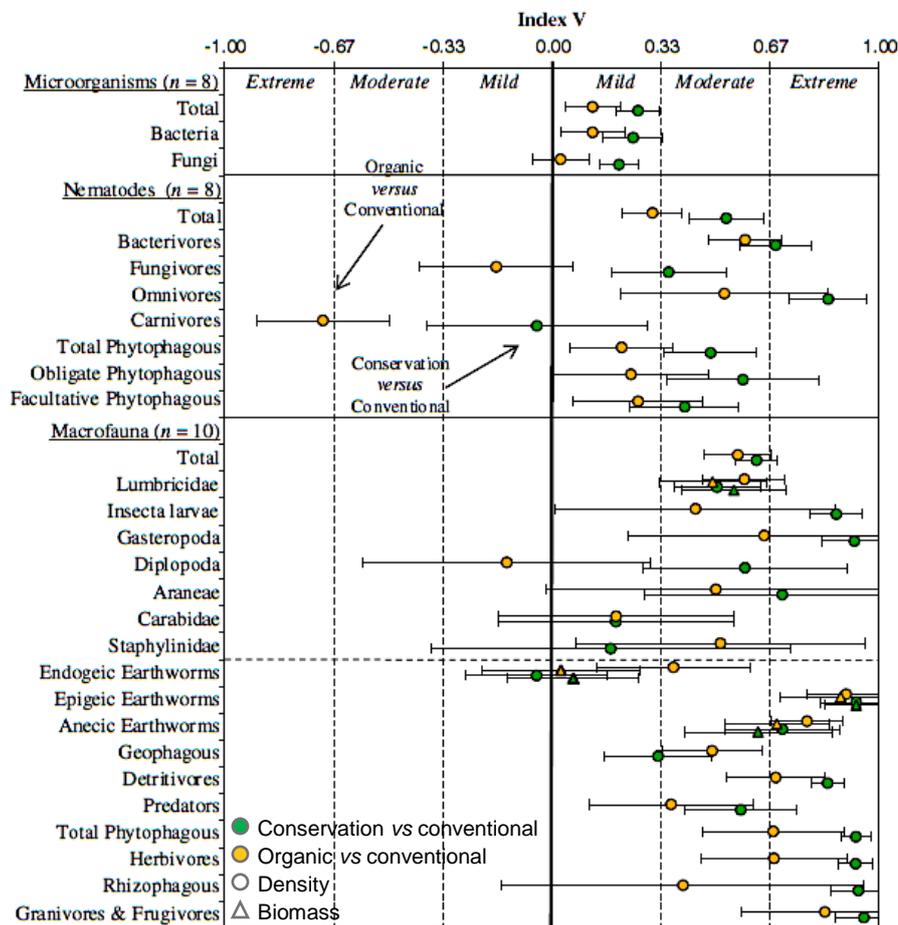


Figure I. 3 Effect of alternative systems on soil microorganisms, microfauna and macrofauna. Index V (mean and 95% confidence interval) represents the level of response of soil organisms between alternative and conventional systems. Modified from Henneron et al. (2015).

I.2.4.2 Transitions toward alternative systems

The conversion to an alternative cropping system bears some risks for farmers. During the first years of the transition, the adoption of new techniques (e.g. ending of pesticides application or no-tillage) could lead to the development of weeds and pests causing an important decrease in crop yield and financial issues (Giller et al., 2009; Scopel et al., 2013). The investment in new devices (e.g. disc harrow for superficial tillage, seed drill adapted to direct tillage) represents an additional cost. In addition, the implementation of new practices involves a progressive change in soil properties. The mechanisms occurring during that period of transition and the time required to reach a new equilibrium are poorly documented. In particular, the effect of transition on soil biodiversity and its impact on soil functioning are unclear.

Studies on transitional systems are mainly conducted on the transition from conventional to organic agriculture. Several groups are found to benefit from this transition, with a reported increase in the abundance of nitrogen-fixing bacteria (Shu et al., 2012), fungi (Coller et al., 2022) and macroarthropods (Lundgren et al., 2006; Tsutsui et al., 2018). Variable effects are reported regarding microbial biomass and microarthropod abundance (Werner and Dindal, 1990; Filser et al., 2002; Schrader et al., 2006; Briar et al., 2007; Santos et al., 2012). There is growing evidence that effects of recent organic systems on organisms vary depending on the time since conversion and on applied practices during the first years of the transition (Jabbour et al., 2016; Tsutsui et al., 2018; Gareau et al., 2019). In addition, some groups may respond faster than others in relation with their trophic position and body size (Coudrain et al., 2016; Coller et al., 2022). Overall, the changes occurring in soil communities during the transition to organic systems remain unclear, especially for diversity, and the drivers of the observed variability are not fully determined.

Fewer studies focus on the effect of the transition from conventional to no-tillage systems on soil organisms (Stubbs et al., 2004; Pelosi et al., 2016; Zhang et al., 2022). Benefits were reported on earthworm density in the first years of the transition but not at long-term (Stubbs et al., 2004; Pelosi et al., 2016). However, the transition to real conservation systems (i.e. not only no-tillage) is poorly investigated, especially in Europe where it is not the dominant alternative. Furthermore, the growing demand for organic products and the potential ban of glyphosate in Europe in the coming years also drive conversion from conservation to organic systems. Yet, we are not aware of studies investigating this transition and its effect on soil biodiversity.

The current lack of knowledge on the effect of the transition prevents to provide adapted support for farmers during the transition process. Improving the knowledge on the mechanisms occurring during the first years of transition may help to limit the disengagement of farmers and to justify the need for some financial compensation during these years.

I.2.4.3 Knowledge gaps to address on alternative cropping systems

To synthesize, the current research gaps on the effects of cropping systems on soil biodiversity result from a lack of studies on 1) the soil biodiversity in alternative cropping systems (i.e. organic, conservation), especially on conservation systems that are understudied in temperate areas, 2) the changes occurring in soil communities during the transition from conventional to alternative systems or from one alternative to another, and on 3) the simultaneous assessment of the density and diversity of multiple taxa of soil organisms and associated soil functions in agricultural soils.

Overall, the variability in the density and diversity of soil organisms in alternative cropping systems is large and remains poorly understood. This is partly due to the large panel of practices applied by farmers even within a similar system type. This variability is often not compensated by the number of sampling points that are limited by various parameters (i.e. time- and labor-consuming measurements and cost of analyses). Further studies could bring additional insight on the effects of alternative systems on different groups of soil organisms and the associated functions under a variety of contexts. Furthermore, the choice of methods applied to characterize cropping systems and soil biodiversity have an important role in the interpretation of the results and the management of the variability.

I.2.5 Methods to characterize cropping systems and soil biodiversity

This part aims to give some insight on the way cropping systems and soil biodiversity can be characterized in order to better describe disturbances caused by agricultural practices, and the effect of these disturbances on soil organisms and associated functions.

I.2.5.1 Characterization of cropping systems

Cropping systems can be characterized by different approaches. In most cases, studies assess the effect of crop management on soil biodiversity either by considering a single practice (e.g. tillage) or by distinguishing between the main existing cropping systems (i.e. conventional, organic, no-till or conservation systems). It remains unclear whether these approaches are relevant to understand the effects of practices on soil properties, functioning and biodiversity as both present some advantages and some drawbacks.

On the one hand, focusing on a single practice can help to better understand the specific effect of that practice on soil biodiversity. However, it provides a partial description of the cropping system and does not take into account interactions between practices. For instance, tillage

associated to organic fertilization may benefit microbial biomass and microfauna density deeper in soil (Treonis et al., 2010). Furthermore, the description of applied practices is often partial and does not take into account all the parameters driving their effect on soil biodiversity. For instance, the effect of tillage is generally investigated by comparing broad categories such as conventional, reduced and no-tillage. These categories do not take into account parameters that govern the intensity of soil disturbances such as tillage depth (deep or surface tillage), frequency (number of machinery passages per year or in the crop rotation) and associated devices (e.g. mouldboard plough, rotary harrow). Similarly, pesticide applications are considered as absent or present in most cases. Chemical disturbances due to pesticides depend on the targets of the products (e.g. herbicides, fungicides, insecticides, molluscicides) and on the application parameters (e.g. dose, number, frequency and timing of application). Finally, the description of the fertilization generally relies on the comparison between organic and mineral fertilizer application, but without including the type of applied mineral (i.e. synthetic product) or organic fertilizers (e.g. manure, compost), the parameters of application (i.e. dose, number of inputs, frequency), or the presence of other organic matter inputs such as crop residues.

On the other hand, the comparison between the main types of cropping systems, such as conventional, organic and conservation systems can be useful to investigate the distinct effects of physical and chemical disturbances on soil. Indeed, organic and conservation systems rely respectively on lower chemical and physical disturbances than conventional systems. Yet, this approach does not take into account the diversity and the intensity of applied practices within these systems. These broad definitions conduct to a large variability within each category, which can hide the overall effect of individual cropping systems on biodiversity. This may not be an issue when comparing cropping systems from an experimental site, as in the vast majority of studies. Nonetheless, this might be problematic for comparing systems under real conditions where practices are supposed to be more variable.

In the recent years, agronomists highlighted the usefulness of indicators describing more finely the practices applied within a cropping system. For instance, indicators were developed to assess the land-use intensity (Armengot et al., 2011), the overall sustainability of agronomic systems (Bockstaller et al., 2015) or to improve the classification of cropping systems (Büchi et al., 2019). In their recent study, Büchi et al. (2019) stated that indicators have the potential to unveil the effect of cropping systems on yield and soil properties. In addition, indicators of practice intensity could be particularly useful to better estimate soil disturbances caused by cropping systems and the associated effects on soil organisms. The advantage of indicators is that they allow to take into account numerous practices and to compare their effects. They can in particular combine several practices related to the same type of disturbance (e.g. surface and deep tillage disturbances). In soil ecology, some authors already expressed their interest for the use of

indicators describing the intensity and frequency of soil disturbances, in order to compare the effect of different managements on soil organisms (Gareau et al., 2019; Masin et al., 2020). The use of a gradient of intensification and the comparison of practice intensity were also suggested as good approaches to understand the link between management intensity, soil biodiversity and ecosystem functions (Giller et al., 1997). Indicators provide one value for each plot instead of grouping them per system, thus switching from a discrete to a continuous variable and enabling to observe a gradient of practice intensity. This is especially relevant in soil ecology where the number of collected samples is often a limiting factor for statistical analyses. However, we found few studies using indicators developed by agronomist to assess the effect of cropping system on soil organisms (Ferraro and Ghera, 2007; Masin et al., 2020).

Indicators should be easy to obtain and should help to differentiate cropping systems. In addition, complex indicators are often correlated with simple ones, hence showing that simple indicators (e.g. number of pesticide applications) may be sufficient to assess applied practices (Büchi et al., 2019). From an agronomic point of view, indicators have to be computed considering all the practices occurring during the crop cycle. However, ecological studies aim at assessing the relations between practices and biodiversity observed at the time of sampling. In that case, there is no interest in considering practices occurring after the sampling date, but it can be relevant to consider all practices occurring from the beginning of current or previous crop cycle to the sampling date. In that context, indicators of practice intensity seem highly relevant to assess the effect of cropping systems on soil biodiversity and functioning. However, this approach requires having access to the history of applied practices on each plot. This information can be easily accessible in experimental plots, which involves the vast majority of studies. However, in real conditions, accessing to the list of applied practices requires to conduct surveys with farmers.

I.2.5.2 Characterization of soil biodiversity

Soil biodiversity is the diversity of organisms, fauna or microorganisms, living in soil during at least part of their life cycle. There are multiple ways to characterize biodiversity (Cadotte et al., 2011). It is commonly assessed through taxonomic diversity, considering the number of taxa (e.g. class, order, species) of soil organisms and their abundance, density or biomass. Species richness represents the phenotypic and genetic diversity within an ecosystem and gives a global vision of the biodiversity. However, assessing species richness requires specific identification skills, which can be a limiting factor as some skills are being lost (e.g. Collembola). In addition, there is a wide diversity of organisms in soil, thus it is difficult to take them all into account. A first way to overcome this limit is to focus on bioindicator species. Bioindicators are organisms that inform on the state and on the functioning of an ecosystem and can reveal specific effects when exposed to

natural or anthropic stress (Bispo et al., 2009). Microorganisms, Collembola and earthworms are considered as good bioindicators of perturbations in agricultural areas (Bispo et al., 2009; Pérès et al., 2011; Socarrás and Izquierdo, 2014; Schloter et al., 2018). Furthermore, groups of species are frequently used in soil ecology, the most common being eco-morphological groups of Collembola and ecological categories of earthworms. These groups were defined arbitrarily based on the available knowledge on the activity they performed in soil or on their environmental preferences. Collembola are divided in three eco-morphological groups, namely euedaphic, hemiedaphic and epedaphic, depending on their adaptation to the soil environment (Gisin, 1943; Parisi et al., 2005). The main ecological categories of earthworms within the seven existing categories are anecic, endogeic and epigeic (Bouché, 1972; Bottinelli et al., 2020). Earthworm ecological categories are often reported as functional groups in the literature. However, this classification is currently being questioned because few studies have focused on ecological group-function relations (Bottinelli and Capowiez, 2021).

Functional groups help to determine the overall effect of biodiversity on the soil functioning. In particular, trophic groups (e.g. detritivores, predators) allow for the assessment of the functions that organisms play in soil through their feeding activity. The characterization of trophic groups relies on the knowledge available on their feeding habits. The knowledge regarding the feeding habits of soil organisms were recently synthesized and can be used to infer trophic relations in soil (Potapov et al., 2022). Various trophic groups interact within the soil food web. The assessment of the soil food web is particularly useful to understand indirect effects of trophic interactions on soil, as well as the direct and indirect effects of disturbances on multiple groups of interacting soil organisms. Further studies are required on soil food webs, in particular in the light of novel techniques holding potential for soil ecologists (Eisenhauer et al., 2015).

Functional traits allow characterizing individuals through their intrinsic characteristics (i.e. morphological, physiological, phenological or behavioral) rather than their taxonomic affiliation (see Box 3 and Figure I.4). In the last decades, they have been increasingly used for soil organisms, especially for invertebrates (Hedde et al., 2012; Pey et al., 2014; deCastro-Arrazola et al., 2022) and more recently for microorganisms (Nguyen et al., 2016; Romillac and Santorufo, 2021). Functional traits are reported to complement the taxonomic approach to describe soil biodiversity (Pey et al., 2014). Traits of microorganisms are considered at the Operational Taxonomic Unit (OTU) level for genomic traits and through direct observations on strain growth. Traits on soil invertebrates are usually considered at the species level (i.e. one trait value per species). Fewer studies conducted traits measurements on individuals (Ribera et al., 2001; Fournier et al., 2012; Moretti et al., 2017). Trait values can vary between individuals of the same species, especially for continuous traits such as body length (Figure I.4).

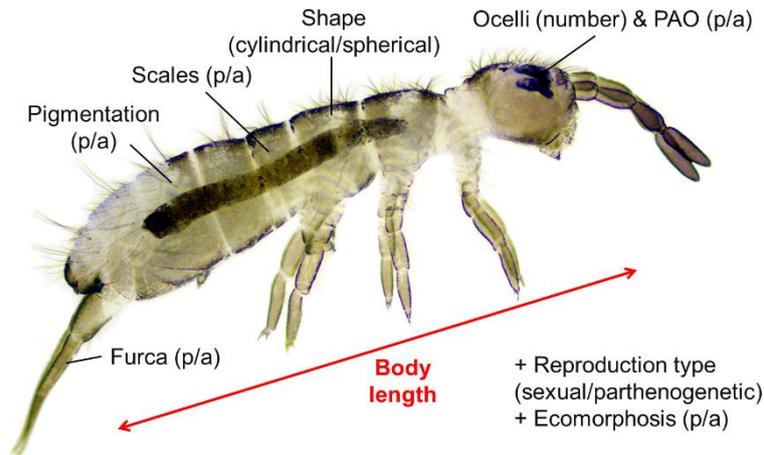


Figure I. 4 Example of functional traits measured on Collembola. Trait attributes are presented in parenthesis. p/a: presence/absence. PAO: post-antennal organ.

Traits of soil organisms are increasingly used to understand species responses to disturbances such as the ones caused by agricultural practices (Hedde et al., 2012; Pey et al., 2014; Salmon et al., 2014). The relations between agricultural practices and traits were mostly studied for ground beetles (Cole et al., 2002; Liu et al., 2012; Boinot et al., 2019; deCastro-Arrazola et al., 2022), and in a lesser extent for Collembola (Santorufu et al., 2015; Martins da Silva et al., 2016; Maisto et al., 2017) and earthworms (Decaëns et al., 2011; Pelosi et al., 2016; Frazão et al., 2019). In order to assess the effects of disturbances, traits should be selected based on their potential to indicate response to these disturbances (e.g. tillage, pesticides) (Petchey and Gaston, 2006). Furthermore, a more controversial use of traits is to assess biodiversity effect on ecosystem functions, thus considering effect traits rather than response traits. Indeed, functional diversity was reported to be more relevant than species richness to study the effect of biodiversity on ecosystem functioning (Coulis et al., 2015) and several studies found species richness to have little or no relation with ecosystem functions (Heemsbergen et al., 2004; Reiss et al., 2009). In particular, the use of functional traits could be of a great help to better understand the effect of agricultural practices on ecosystems functions (Wood et al., 2015). Thus, there is a growing interest on the use of functional traits for the assessment and management of ecosystem services.

Functional traits are used to estimate the functional diversity within a community. This is achieved by computing several functional indexes based on traits such as functional richness, evenness or divergence (Lavorel et al., 2008; Villéger et al., 2008).

Overall, the measurement of biodiversity in cropping systems must not be reduced to species richness, especially as cropping systems are reported to have a low number of species. Functional traits and diversity could provide additional insight on the effect of disturbances due to practices on soil biodiversity. In addition, the interactions between trophic groups could help to understand to overall changes occurring in the soil community and their impacts on soil functioning.

Box 3. Definition and history of functional traits

Traits-based approaches were first developed by plant ecologists (Lavorel and Garnier, 2002; McGill et al., 2006) and applied to freshwater ecosystems (Usseglio-Polatera et al., 2000) and to animal ecology (Vandewalle et al., 2010). Traits can be grouped in different categories, including response and effect traits. Effect traits are individual characteristics that can affect ecosystem functioning (Violle et al., 2007). Functional traits, also named response traits, are individual characteristics reflecting the relation between an organism and its environment. A general definition of functional traits is “any traits which impacts fitness indirectly *via* its effects on growth, reproduction and survival” (Violle et al., 2007). This definition was extended for soil invertebrates as “any morphological, physiological, phenological or behavioural (MPPB) feature measurable at the individual level, from the cell to the whole-organism level, without reference to any other level of organization” (Pey et al., 2014). It does not include performance traits (e.g. biomass and survival) or ecological preferences (e.g. optimum soil moisture). The most encountered traits for soil invertebrates are body size and body mass (de Bello et al., 2010; Pey et al., 2014). However, considering body size as a species trait is controversial as it is also considered as a “population parameter derived from a trait” (Pey et al., 2014). This could be override by trait measurements on each individual. Traits for soil invertebrates are available in dedicated databases such as BETSI (<http://www.reseau-tebis.fr>) or edaphobase (Burkhardt et al., 2014).

Functional traits were developed more recently in microbial ecology, first for fungi (Nguyen et al., 2016; Zanne et al., 2020) and then for bacteria (Cébron et al., 2021; Romillac and Santorufo, 2021). Bacterial functional traits are defined by Romillac and Santorufo (2021) as “any genomic, morphological, or physiological characteristics potentially measurable at the individual level (without reference to the environment or any other level of organization) and that impact on the organism’s fitness”. This definition excludes the responses to environmental variables and environmental preferences (i.e. conditions for optimal growth). However, not all studies use the same definition and bacterial traits may include preferences traits (Cébron et al., 2021). Bacterial traits are assessed from isolated bacterial strains (i.e. pure culture bacteria). Thus, contrary to invertebrates, the attribution of functional traits is currently limited to bacteria that can be cultivated in laboratory, which is not the case for a large part of bacteria encountered in soils. Databases are available on fungal traits such as FUNguild (Nguyen et al., 2016) and on bacterial traits such as BactoTraits (Cébron et al., 2021).

I.3 Objectives of the thesis

The main objective of this PhD thesis is to characterize the effect of stable and transitional alternative cropping systems, compared to conventional systems, on soil organisms and associated ecosystem services. This relies on the assessment of multiple soil organisms (macrofauna, mesofauna, microorganisms), on their interactions, and on soil functions (litter decomposition, C and N mineralization, soil structural stability) involved in soil chemical and physical fertility and in climate regulation. The overall aim of this thesis is on the short-term to improve our knowledge on soil ecology in an agricultural context, and on the long-term to provide additional support for the development of sustainable agroecosystems relying on strong soil biodiversity and functioning.

This work aims to answer different research questions and hypotheses arising from the state of the art described above:

- What are the effects of alternative cropping systems on soil biodiversity and soil functions?
 - Hypothesis 1: Density and diversity of soil organisms are higher under alternative cropping systems (conservation, organic) and community composition vary compared to conventional systems, resulting from lower physical or chemical soil disturbances.
 - Hypothesis 2: Alternative systems promote a better soil functioning through the benefits of lower physical and chemical disturbances on soil organisms and the functions they perform in soil. Different systems may however promote different functions.

- How do soil organisms and soil functions respond to the transition toward alternative systems?
 - Hypothesis: Density, diversity and community structure of soil organisms vary depending on the stage of the system (i.e. stable or in transition). Systems in transition present more variability in the density and diversity of soils organisms, and different features than stable systems due to a progressive change in applied practices and soil properties.

- Which agricultural practices have the largest impact on soil organisms?
 - Hypothesis 1: An increase in tillage and pesticide treatment intensities have an overall negative effect on soil organisms due to direct and indirect damage, with a higher impact of tillage than pesticide treatments. An increase in fertilization intensity has positive effects on soil organisms at long-term mostly through an increase in the food resource.
 - Hypothesis 2: Practice intensity influences soil trophic groups directly and through effects on trophic interactions within the soil food web. Thanks to lower practice intensity, alternative systems have the potential to promote trophic interactions.

- Which approaches can best characterize the effects of cropping systems on soil biodiversity and soil functioning?
- Hypothesis 1: Indicators of practice intensity allow characterizing the effect of cropping systems and associated disturbances on soil organisms and soil functioning.
 - Hypothesis 2: Functional traits of soil organisms provide more precise insight on soil biodiversity and on its relation to soil disturbances than the sole use of taxonomic identification.

In order to answer these questions, the thesis is structured in six chapters (Figure I.5). They are preceded by a description of the study sites and experimental design, at the end of this introduction, and followed by a part including general discussion and conclusion.

In the first three chapters, we characterize the effect of cropping systems on soil organisms, with a focus on soil mesofauna, macrofauna and microorganisms respectively. To that aim, we assess conjointly 1) the effect of cropping systems by comparing results obtained at the system level (conventional, organic and conservation agriculture; stable or transitional systems) and using indicators of practice intensity (tillage, pesticide treatments, fertilization) and 2) soil biodiversity using taxonomic diversity and functional diversity based on the use of functional traits.

In the fourth chapter, we synthesize the overall effects of alternative and transitioning systems on the soil community compared to conventional systems, and we assess the effects of practice intensity on trophic groups and their interactions within the soil food web.

In the last two chapters, we discuss about the effect of cropping systems on several soil functions driven by soil organisms. First, the fifth chapter corresponds to a meta-analysis on litterbag experiments in cropping systems and on the effect of soil fauna on litter decomposition. Then, in the sixth chapter, we present results on the measurement of some soil functions performed by soil organisms (litter decomposition, C and N mineralization, soil structural stability) and we discuss about the influence of cropping systems on these functions.

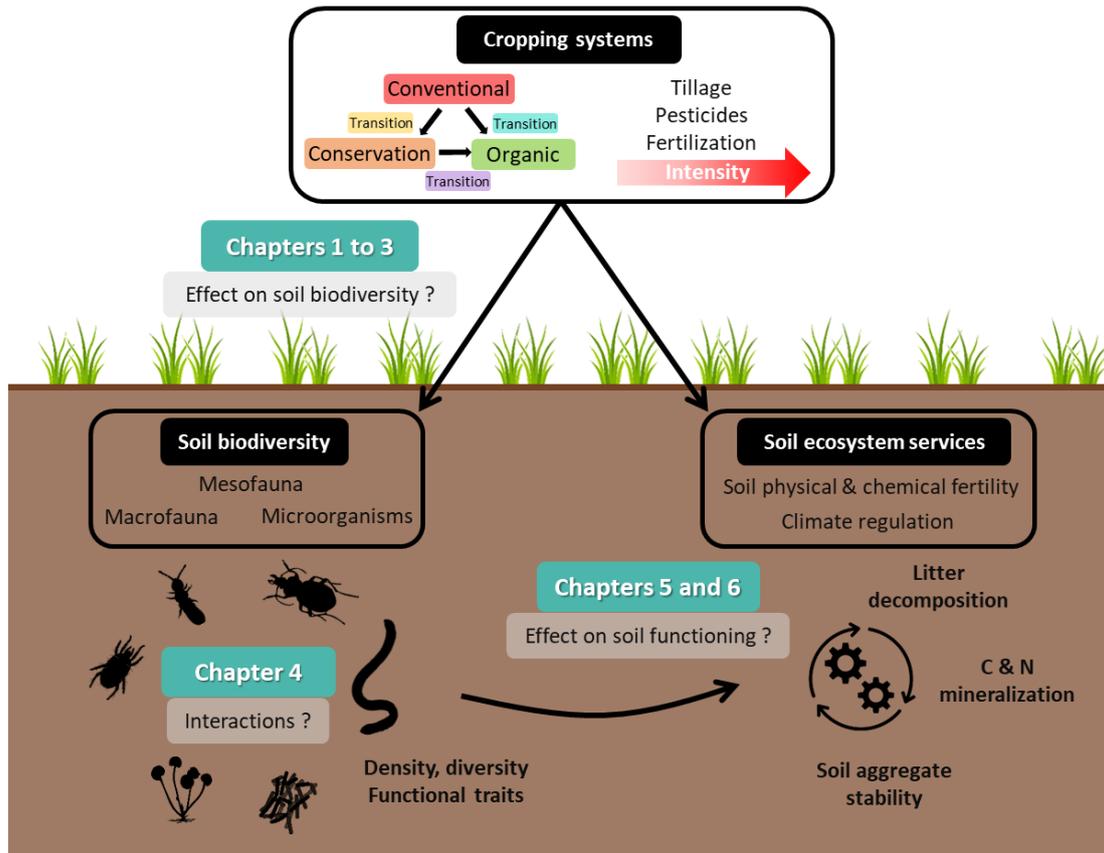


Figure I. 5 Graphical abstract of the thesis presenting the focus of the six chapters.

I.4 Characterization of the study area and experimental approach

The results of this thesis were obtained by combining measurement conducted on the field and in the laboratory. Soil and biodiversity samples were collected from 21 plots in autumn 2020 and in autumn 2021. The objective was to observe the soil organisms at the beginning and a year after wheat cultivation under different cropping systems. The following part presents common features of all the chapters of the thesis, namely the main characteristics of the study area, description of applied practices, sampling design and soil properties at the study sites. More specific analyses are described in the dedicated chapters.

I.4.1 Study sites

The study area comprised 21 plots located in the Paris basin, France, within the Yvelines, Eure-et-Loir and Essonne departments (Figure I.6). The Paris basin is an important agricultural area in France characterized by an intensive cereal production, mostly wheat, and by an open-field landscape. Plots were selected from real farms to take into account the potential variability of practices within different cropping systems. Most plots were owned by farmers of the Scarabee network, which is a network created by the INRAE (Agronomie Unit, Palaiseau) in order to implement and study experimental flower strips in association with various agricultural practices. As not enough plots were available for each system, we additionally selected one experimental plot (La Cage experimental, Versailles, INRAE) and four other plots owed by farmers out of the network. One of the main issues was finding farms recently converted to conservation agriculture, hence for this system two plots are owned by the same farmer.

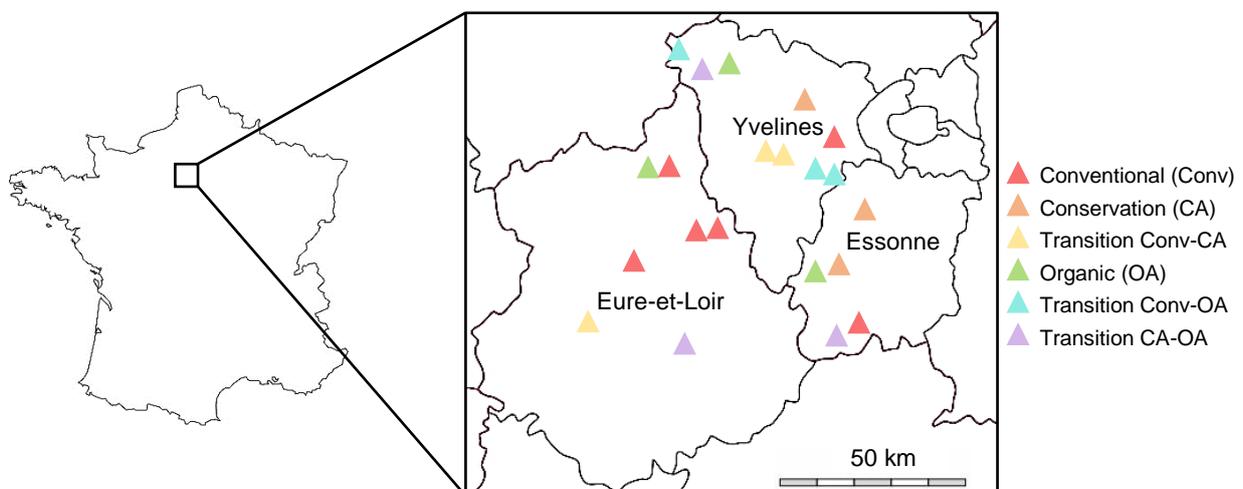


Figure I. 6 Map of the sampling area located in the Paris basin, France. Triangles represent study sites. Colors correspond to the cropping system.

Climate is temperate with mean annual temperatures of 11°C and mean annual precipitations of 600-700 mm. Total precipitations and average min-max temperatures over the study period were 77-91 mm and 5-16°C in 2020, and 84-110 mm and 3-17°C in 2021 (Meteociel). The year 2020 was marked by a particularly hot and dry summer, whereas summer 2021 was the coolest and rainiest since 2014. The autumn was also slightly warmer and humid in 2020 than 2021 (MeteoFrance).

All plots were cropped with winter wheat during the 2020-2021 crop cycle. The date of wheat sowing was variable as conservation and conventional plots are sowed earlier (from October 1st to October 22th) than organic plots (from October 26th to November 24th). Late sowing is a current practice in organic agriculture that aims to compensate for the absence of pesticide treatments. The sowing is occurring just before the arrival of winter cold and rain. It enables to conduct late tillage in order to remove weeds and to prevent pest species attacks that may flourish in autumn. During the 2021-2022 crop cycle, plots were cropped with either cereals (wheat, barley, rye), cruciferous plants (mustard, rapeseed) or cover crops (clover, alfalfa, mixed crops) (Table I.2). In France, it is compulsory to cover the soil during winter, however three plots were reported as “bare” at the time of sampling due to dry unfavorable weather conditions in summer preventing cover establishment. Another reason is the late decision of farmers regarding the choice of the next crop, which conducted us to sample before sowing in a few cases.

Table I. 2 Location, crop and cropping system of the study plots. Age corresponds of the number of year since conversion to the system in 2020. Conv: conventional agriculture, CA: conservation agriculture, OA: organic agriculture, Conv-CA, Conv-OA and CA-OA: initial-recent system transitions (≤ 3 years).

Plot	Region	Crop 2019	Crop 2020	Crop 2021	System	Age
A1	Eure-et-Loir	rapeseed	wheat	wheat	Conv-CA	3
A2	Essonne	faba beans	wheat	cover	CA	17
A3	Essonne	alfalfa	wheat	wheat	OA	20
A4	Yvelines	triticale, peas	wheat	bare	OA	19
A5	Eure-et-Loir	rapeseed, flax, peas	wheat	barley	Conv	20
A6	Essonne	lentil	wheat	bare	CA-OA	2
A7	Yvelines	cover	wheat	rye	Conv-OA	2
A8	Essonne	rapeseed	wheat	mustard	CA	7
A9	Yvelines	faba beans	wheat	rapeseed	Conv	20
A10	Eure-et-Loir	rapeseed	wheat	alfalfa	Conv	20
A11	Yvelines	rapeseed	wheat	cover	OA	20
A12	Yvelines	rapeseed	wheat	cover	Conv	20
A13	Yvelines	maize	wheat	rye, lentil	CA-OA	2
A14	Yvelines	barley	wheat	cover	Conv-CA	3
A15	Yvelines	faba beans	wheat	wheat	Conv-CA	3
A16	Essonne	rapeseed	wheat	mustard	Conv	20
A17	Yvelines	barley	wheat	rapeseed	CA	10
A18	Yvelines	alfalfa, wheat	wheat	bare	Conv-OA	3
A19	Yvelines	grass	wheat	clover	Conv-OA	2
A20	Eure-et-Loir	triticale, faba beans	wheat	cover	CA-OA	2
A21	Eure-et-Loir	rapeseed	wheat	barley	Conv	20

I.4.2 Cropping systems and applied practices

The study is conducted on six cropping systems determined according to three types of agriculture (conventional, organic, conservation) and two stages (stable or in transition) (Figure I.7, Table I.2). Stable systems are established for seven to more than 20 years in conventional (Conv, $n = 6$ plots), organic (OA, $n = 3$) or conservation agriculture (CA, $n = 3$). Systems in transition are converted to alternative systems since two to three years, either from conventional to organic (Conv-OA, $n = 3$), conventional to conservation (Conv-CA, $n = 3$) or conservation to organic agriculture (CA-OA, $n = 3$).

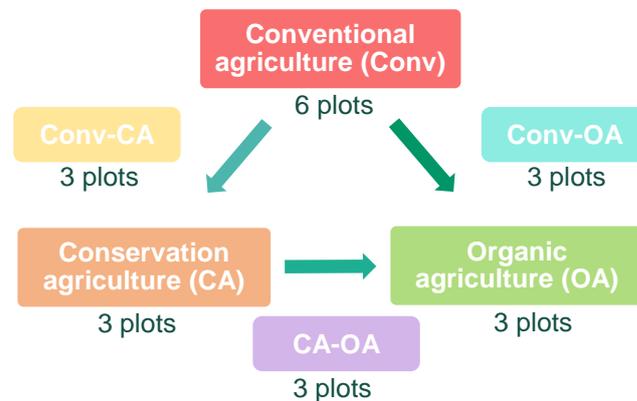


Figure I. 7 Studied cropping systems and associated number of sampled plots.

A survey was conducted among farmers to determine the practices applied to each plot. Information was collected on crops (crop type, rotation, variety, intercropping), tillage (deep and surface tillage, machines), fertilization (number of mineral and organic inputs, dose of mineral nitrogen, type of product, residues exports), pesticide treatments (number and dose of inputs, sprayed surface, products) and on the dates of all operations including seedling and harvest.

Indicators of practice intensity were computed in order to describe the cropping systems and the associated practices in each plot. We decided to follow the methodology and to select indicators proposed by Büchi et al. (2019). From that study, we selected indicators that are easy to obtain, contribute to differentiate cropping systems and do not show strong correlation. When possible, we favored simple indicators over ones that are more complex. Selected indicators belonged to four categories: crop diversification, soil physical disturbances, chemical crop protection and organic matter inputs and nitrogen fertilization (Table I.3). As our objective was to assess the effect of practices on soil organisms collected in autumn, we considered practices occurring in the previous crop cycle. The 2020 and 2021 indicators were computed using practices occurring from the soil preparation and sowing in autumn 2019 or 2020 and until the sampling date in autumn 2020 or 2021 respectively (Figure I.8). In addition, several indicators

accounted for practices applied over the last five years, therefore providing a mean to better account for the difference between stable and transitional alternatives systems.

There are two types of indicators: 1) primary indicators of practice intensity represent the intensity of a single practice over the crop cycle (1 y) or the average crop rotation length (5 y), and 2) composite indexes of practice intensity that are computed by averaging several primary indicators from the same category (Table I.3).

Table I. 3 Primary indicators and composite indexes of practice intensity used to characterize cropping systems. Composite indexes are given in bold. Time is the time span in which data were collected to compute the indicator. Indicators and indexes are adapted from Büchi et al. (2019).

Category	Name	Time	Content
(a) Crop diversification	nbCrop	5 y	Number of different crops in the last 5 years
(b) Soil physical disturbances (tillage)	nbDTill	1 y	Number of deep tillage (i.e. ploughing, > 15 cm depth)
	nbSTill	1 y	Number of surface tillage (i.e. all shallow tillage activities < 15 cm depth such as mechanical weeding, stubble incorporation, stones removal, seedbed preparation and rolling)
	soilP	5 y	Type of tillage in the last 5 years, calculated as the average of annual tillage weight (plough = 0.5, reduced tillage = 3, no-till = 5)
	Itill		Average of tillage indicators: (nbDTill + nbSTill + (1 - soilP)) / 3
(c) Chemical crop protection (pesticide treatments)	nbHerb	1 y	Number of herbicide treatments
	nbFung	1 y	Number of fungicide treatments
	nbInsect	1 y	Number of insecticide treatments
	Itreat		Total number of pesticide treatments: herbicides, fungicides, insecticides, molluscicides and growth regulators
	TFI		Treatment frequency index comprising herbicides, fungicides, insecticides, molluscicides and seed treatments applied during the crop cycle (from sowing to harvest)
(d) Fertilization	nbOrg	5 y	Number of organic amendments in the last 5 years
	qminN	1 y	Quantity of mineral nitrogen fertilizers inputs (kg N.ha ⁻¹)
	resExp	5 y	Number of residues exportation in the last 5 years
	Ifertil		Average of fertilization indicators: (nbOrg + qminN + resExp) / 3

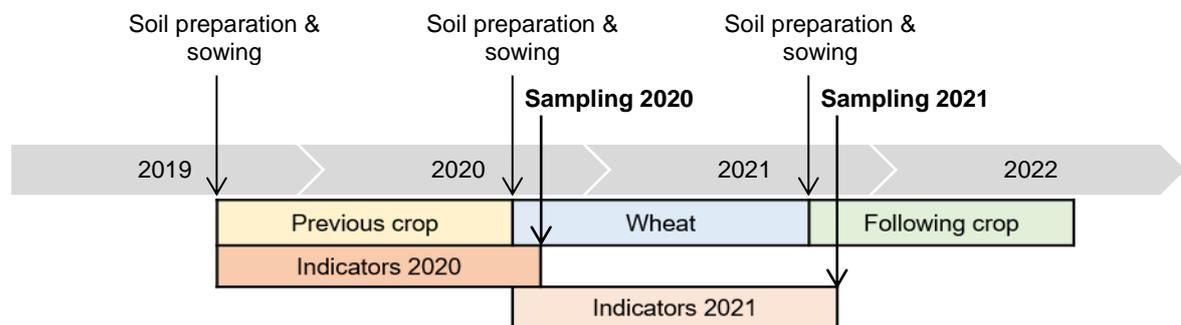


Figure I. 8 Timeline used to define indicators of practice intensity in 2020 and in 2021. Only one-year indicators are represented. Five-year indicators were computed similarly by accounting for the five crop cycles preceding the sampling.

Composite indexes can be computed using different methods. Additive aggregation simply consists in calculating the mean of normalized indicators (values between 0 and 1). Each indicator must be given a direction in order to represent an increase in the management intensity. Another method to compute composite indexes is by conducting a PCA on the selected indicators, after their normalization, and then to use the first components of PCA as the index value for each site. With this method, it is not possible to account for the direction of practices. According to Büchi et al. (2019), this is useful when exploring a dataset having no ideas of the relation between indicators. However, when the objective is to create a unique index and if the direction of each indicators can be hypothesized, the additive aggregation is preferable. In this study, we used the additive aggregation method to compute composite indexes on soil physical disturbances, chemical crop protection and fertilization following Büchi et al. (2019). First, primary indicators were all normalized to obtain values between 0 and 1:

$$I_{norm} = \frac{(I_i - I_{min})}{(I_{max} - I_{min})}$$

where I_{norm} is the normalized primary indicator, I_i is the indicator value at the plot i , I_{min} and I_{max} are the minimal and maximal indicator values within all plots. Then, the composite indexes of tillage intensity (I_{till}) and fertilization intensity (I_{fertil}) were computed by an additive combination of the normalized indicators such as:

$$I_{till} = \frac{nbDTill_{norm} + nbSTill_{norm} + (1 - soilP_{norm})}{3}$$

where I_{till} is the composite index for tillage intensity and $nbDTill_{norm}$, $nbSTill_{norm}$ and $soilP_{norm}$ are respectively the normalized values of the number of deep tillage, surface tillage and average type of tillage over five years, and:

$$I_{fertil} = \frac{nbOrg_{norm} + qminN_{norm} + resExp_{norm}}{3}$$

where I_{fertil} is the composite index for fertilization intensity and $nbOrg_{norm}$, $qminN_{norm}$ and $resExp_{norm}$ are respectively the normalized values of the number of organic matter inputs, quantity of mineral N inputs ($\text{kg N}\cdot\text{ha}^{-1}$) and number of residues exportation. The composite index for pesticide treatment intensity (I_{treat}) was the sum of the total number of pesticide treatments applied over the period such as:

$$I_{treat} = nbTreat_{norm}$$

where I_{treat} is the composite index for pesticide treatment intensity and $nbTreat_{norm}$ is the normalized value of the total number of treatments (including herbicides, fungicides, insecticides,

molluscicides, growth regulator). Each product was considered as a separated treatment even when applied together with other products.

In addition, the treatment frequency index (TFI) was computed. The TFI is a common index used by farmers and agronomists in order to assess the intensity of treatments over a crop cycle (Jørgensen and Kudsk, 2006). It was calculated for each treatment (herbicides, fungicides, insecticides, molluscicides, growth regulators, seed treatments), but not for adjuvants, such as:

$$TFI_{product} = \frac{Applied\ dose}{Recommended\ dose} \times \frac{Treated\ surface}{Field\ surface}$$

Then, the total TFI over the crop cycle was obtained with the sum of the TFI of all products applied over the crop cycle:

$$TFI_{total} = \sum_{seedling}^{harvest} TFI_{product}$$

Whenever the information on a product or on seed treatment was missing, we added a TFI of 1 to the total TFI. However, as we found that I_{treat} and TFI were highly correlated (Spearman correlation, $cor = 0.9$, $P < 0.001$) and as TFI was missing in two plots, we used only I_{treat} for the rest of the study. Values of primary indicators and composite indexes are reported in Table I.4.

As in Büchi et al. (2019), primary indicators made possible to discriminate between the different cropping systems, and separate organic systems from conventional and conservation systems both in 2020 and in 2021 (Figure I.9). This confirms the robustness of this approach.

Furthermore, composite indexes form a continuous gradient of practice intensity within plots. As expected, tillage intensity is very low or absent in conservation systems, while pesticide treatment intensity is at zero in organic systems (Figure I.10). Nevertheless, intensity indexes also reveal effects that were hidden by the classification into systems. First, tillage intensity is not always higher in organic than in conventional plots. Especially, one CA-OA plot has a low tillage intensity (A13), one Conv plot is part of the most tillage intensive plots (A9) while other Conv plots can have a low tillage intensity (e.g. A10 in 2021). Then, the pesticide treatment intensity is highly variable within conventional and conservation plots. One CA plot (A8) has a particularly low treatment intensity. Finally, fertilization intensity is highly variable between and within systems. It may indicate that primary indicators are more efficient than the composite index to assess fertilization. Indeed, organic matter input is higher in organic than in conventional and conservation systems in both years, while mineral nitrogen input is the highest in conventional systems. Intensity indexes are not significantly different between stable and transitioning systems, except for the absence of pesticide treatments right after the transition to organic farming.

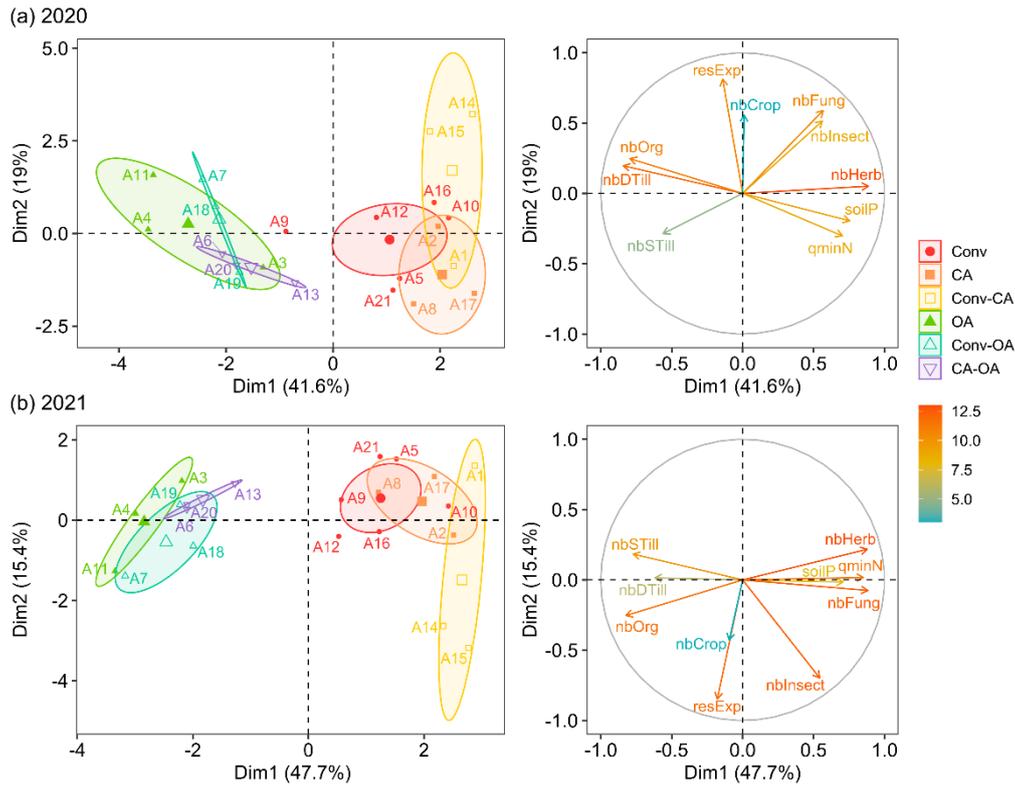


Figure 1.9 PCA on primary cropping practice indicators in 2020 and 2021. Ellipses represent cropping systems. Smaller points are plots and larger points are ellipse centroids. Conv: conventional agriculture; CA: conservation agriculture; OA: organic agriculture; Conv-CA, Conv-OA and CA-OA: initial-recent system transitions.

Table I. 4 Indicators and composite indexes of practice intensity in the 21 studied plots in (a) 2020 and (b) 2021.

	Conv						CA			Conv-CA			OA			Conv-OA			CA-OA		
	A5	A9	A10	A12	A16	A21	A2	A8	A17	A1	A14	A15	A3	A4	A11	A7	A18	A19	A6	A13	A20
(a) 2020																					
nbCrop	3.0	3.0	4.0	2.0	4.0	3.0	4.0	3.0	3.0	3.0	4.0	4.0	3.0	4.0	4.0	4.0	3.0	2.0	3.0	3.0	4.0
nbDTill	0.0	2.0	1.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	2.0	2.0	2.0	2.0	1.0	2.0	0.0	1.0
nbSTill	7.0	8.0	0.0	3.0	7.0	7.0	0.0	2.0	0.0	1.0	0.0	0.0	4.0	9.0	6.0	3.0	3.0	5.0	3.0	6.0	12.0
soilP	3.0	0.5	2.3	1.0	2.5	2.5	5.0	4.6	5.0	3.4	4.2	3.8	2.8	0.5	0.5	0.5	1.5	2.5	1.4	3.0	2.5
Itill	0.3	0.9	0.4	0.5	0.4	0.4	0.0	0.1	0.0	0.1	0.1	0.1	0.4	0.9	0.8	0.8	0.7	0.5	0.7	0.3	0.7
nbHerb	4.0	3.0	8.0	4.0	7.0	5.0	7.0	2.0	10.0	6.0	5.0	5.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
nbFung	1.0	1.0	1.0	1.0	1.0	0.0	1.0	0.0	0.0	0.0	2.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
nbInsect	0.0	1.0	1.0	2.0	3.0	1.0	0.0	0.0	0.0	2.0	2.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Itreat	0.5	0.4	1.0	0.6	0.9	0.5	0.7	0.2	0.9	0.8	0.8	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
TFI	4.5	3.8	7.1	5.3	6.0	3.4	4.4	1.0	4.0	6.4	NA	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
nbOrg	1.0	0.0	0.0	2.0	2.0	0.0	1.0	0.0	1.0	0.0	1.0	2.0	3.0	6.0	6.0	3.0	3.0	4.0	3.0	2.0	2.0
qminN	172	0	162	179	122	139	0	165	163	140	67	0	0	0	0	0	0	0	0	0	0
resExp	0.0	0.0	0.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	4.0	3.0	0.0	0.0	3.0	3.0	3.0	1.0	0.0	0.0	0.0
Ifertil	0.4	0.0	0.3	0.6	0.3	0.3	0.1	0.3	0.4	0.3	0.5	0.4	0.2	0.3	0.6	0.4	0.4	0.3	0.2	0.1	0.1
(b) 2021																					
nbCrop	3.0	4.0	4.0	2.0	5.0	3.0	4.0	4.0	3.0	3.0	4.0	4.0	3.0	4.0	4.0	5.0	3.0	3.0	4.0	3.0	4.0
nbDTill	0.0	2.0	0.0	1.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	1.0	1.0	2.0	1.0	1.0	1.0	0.0	0.0
nbSTill	7.0	5.0	0.0	3.0	3.0	6.0	0.0	0.0	0.0	1.0	0.0	0.0	7.0	7.0	9.0	9.0	4.0	8.0	4.0	6.0	15.0
soilP	3.0	0.5	3.2	1.0	2.5	2.0	5.0	4.6	5.0	3.4	4.6	4.2	2.8	0.5	0.5	0.5	1.5	2.5	1.4	3.0	2.5
Itill	0.3	0.8	0.1	0.5	0.3	0.5	0.0	0.0	0.0	0.1	0.0	0.1	0.7	0.7	0.7	0.9	0.5	0.5	0.5	0.3	0.5
nbHerb	9.0	7.0	9.0	6.0	8.0	8.0	8.0	3.0	7.0	10.0	3.0	6.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
nbFung	2.0	2.0	2.0	1.0	2.0	2.0	2.0	0.0	3.0	4.0	3.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
nbInsect	0.0	1.0	1.0	2.0	1.0	0.0	2.0	0.0	0.0	0.0	2.0	4.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Itreat	0.8	0.8	0.9	0.6	0.7	0.6	0.8	0.2	0.8	1.0	0.5	0.8	0.0	0.1	0.0						
TFI	9.9	12.3	8.9	7.9	8.9	8.1	9.7	1.4	6.6	6.6	NA	NA	0.0	0.9	0.0						
nbOrg	1.0	0.0	0.0	2.0	2.0	0.0	1.0	0.0	1.0	0.0	1.0	2.0	3.0	6.0	6.0	3.0	3.0	4.0	4.0	3.0	2.0
qminN	217	221	178	190	132	249	124	173	94	166	213	183	0	0	0	0	0	0	0	0	0
resExp	0.0	0.0	0.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	4.0	3.0	0.0	0.0	3.0	3.0	3.0	1.0	0.0	0.0	1.0
Ifertil	0.3	0.3	0.2	0.5	0.3	0.3	0.2	0.2	0.2	0.2	0.7	0.6	0.2	0.3	0.6	0.4	0.4	0.3	0.2	0.2	0.2

Conv: conventional agriculture, CA: conservation agriculture, OA: organic agriculture, Conv-CA, Conv-OA and CA-OA: initial-recent system transitions, nbCrop: nb of crops (5 y), nbDTill: nb of deep tillage, nbSTill: nb of surface tillage, soilP: type of tillage (5 y), Itill: tillage intensity index, nbHerb: nb of herbicides, nbFung: nb of fungicides, nbInsect: nb of insecticides, Itreat: pesticide treatment intensity index, TFI: treatment frequency index, nbOrg: nb of organic matter inputs (5 y), qminN: quantity of mineral nitrogen (kg N.ha⁻¹), resExp: nb of residues exportations (5 y), Ifertil: fertilization intensity index.

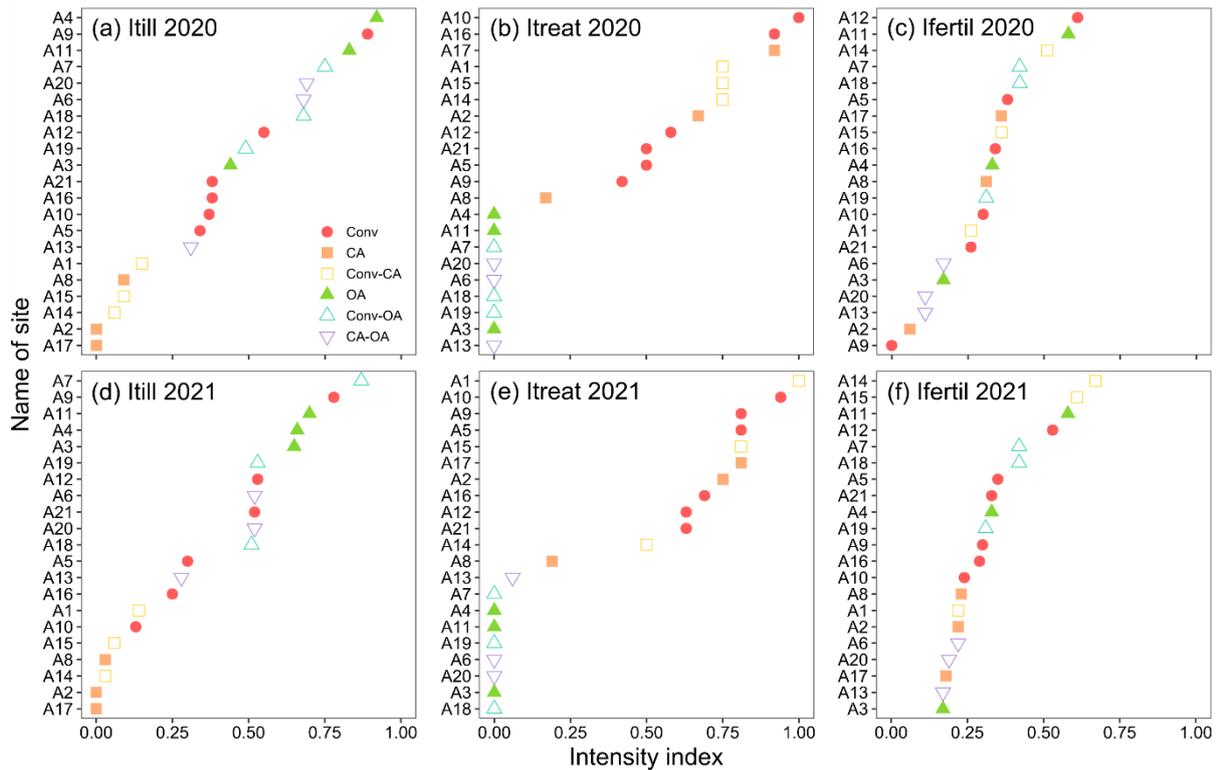


Figure I. 10 Composite indexes of the intensity of tillage (Itill), pesticide treatments (Itreat) and fertilization (Ifertil) in the study plots. Symbols correspond to system groups as reported in the legend. Conv: conventional agriculture; CA: conservation agriculture; OA: organic agriculture; Conv-CA, Conv-OA and CA-OA: initial-recent system transitions.

I.4.3 Experimental design

Samples were collected in the 21 plots for two consecutive years, from October 19th to December 2nd in 2020 and from October 25th to November 22nd in 2021. To enable soil to recover after mechanical disturbances, sampling was performed minimum one week after sowing. In addition, samples were collected between crop rows and outside of tractor traffic tracks. Three replicates were defined in each plot, spaced 25 m from one another and positioned at 25 m from the field margins (Figure I.11).

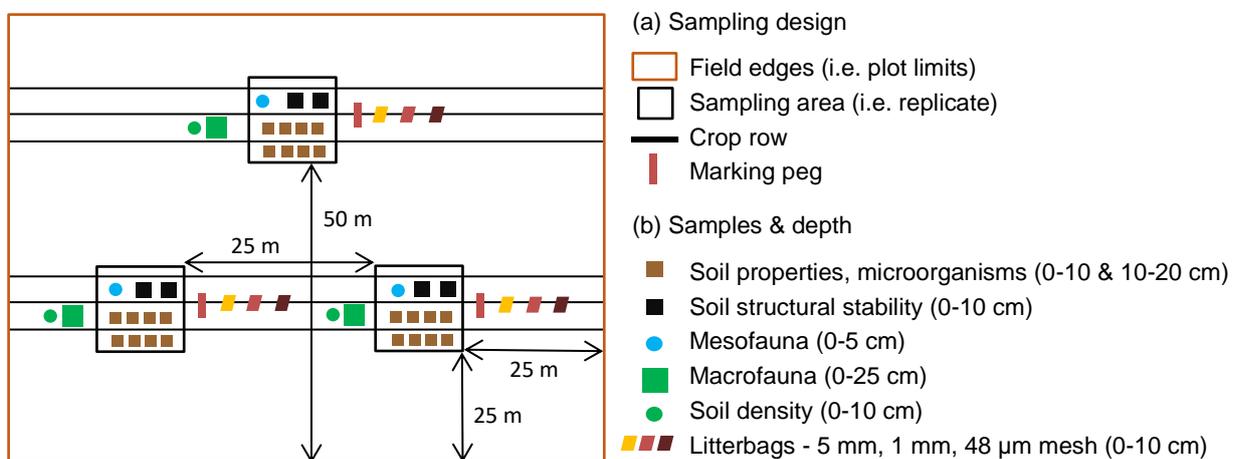


Figure I. 11 Schematic representation of the experimental design.

I.4.4 Soil properties

Composite soil samples were collected at 0-10 and 10-20 cm depth. Eight soil cores obtained with an auger were mixed on each replicate. The soil water content was assessed by drying fresh soil (105°C, 48h). Soil physico-chemical characteristics and bulk densities were only determined in 2020. Soil was sieved at 4 mm, air dried and sent to the INRAE Laboratory of Arras for physico-chemical analyses. The soil bulk density was measured at 0-10 and 10-20 cm depth with soil cores (10 cm in diameter) dried (105°C, 48h) and weighed.

Soil properties are presented in Tables I.5 and I.6. Soils are silty (n = 16) or more silty-clayed (n = 5). One plot (A4) has a higher percentage of clay (426-439 g.kg⁻¹). Soil pH ranges from 5.6 to 8.1 at 0-10 cm depth and from 5.5 to 8.2 at 10-20 cm depth. A rather low pH was observed in A8 (5.5-5.6) leading to an increase in the quantity of aluminum ions in soil solution. The highest pH was observed in A17 (8.1-8.2) where the quantity of carbonates was also high (143-144 g.kg⁻¹) due to the presence of calcareous rocks and fossils. Bulk density ranges from 1.1 to 1.5 at both depths and tends to be higher at 0-10 cm than at 10-20 cm depth in most plots.

Overall, soil properties at 0-10 cm depth in stable conservation systems are different from all the other systems (Figure I.12). In addition, recent conservation systems differ from both conventional and stable conservations systems at 0-10 and 10-20 cm depth (Figure I.12). The C/N ratio is the main variable explaining differences at 0-10 cm.

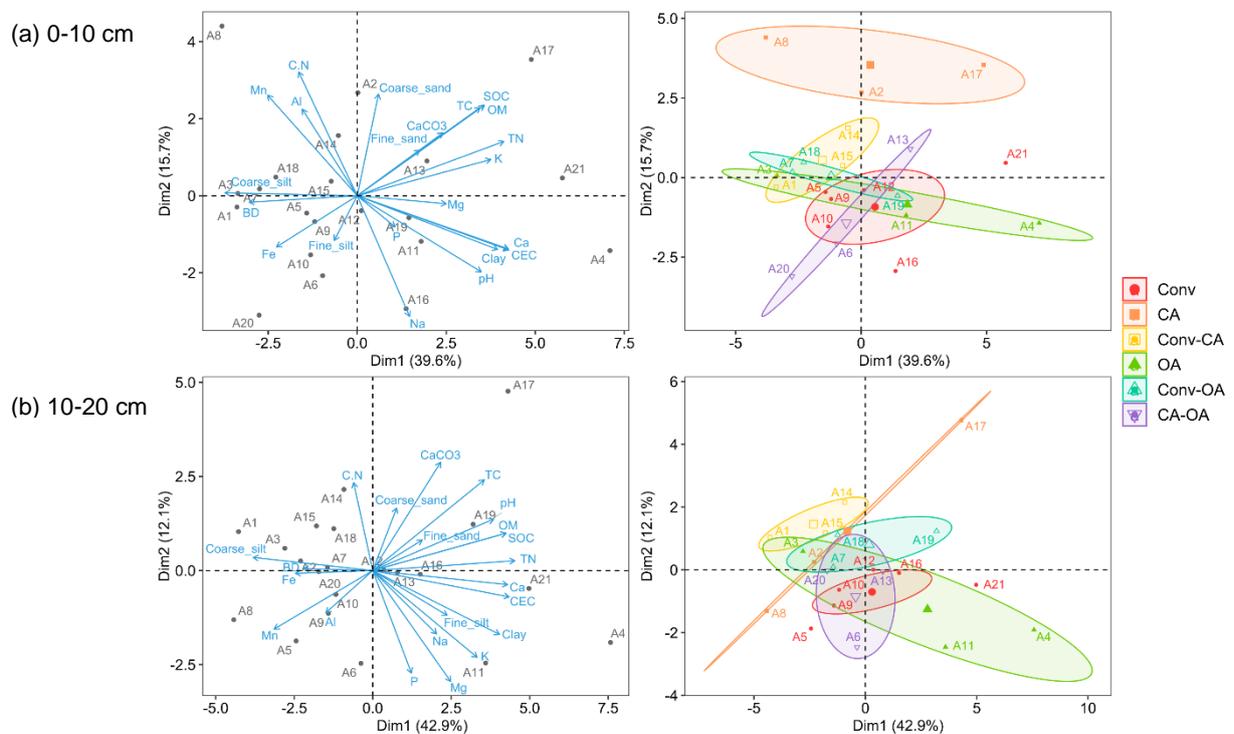


Figure I. 12 PCA on soil parameters in the 21 plots of the study at (a) 0-10 cm and (b) 10-20 cm depth. Conv, CA and OA: conventional, conservation and organic agriculture; Conv-CA, Conv-OA and CA-OA: initial-recent system transitions.

Table I. 5 Soil physico-chemical properties at 0-10 cm depth in the 21 plots of the study. Soil analyses were conducted in 2020, and water content was assessed in 2020 and 2021.

	Clay g.kg ⁻¹	Silt g.kg ⁻¹	Sand g.kg ⁻¹	pH	CEC cmol+.kg ⁻¹	SOC g.kg ⁻¹	TN g.kg ⁻¹	C/N	CaCO3 g.kg ⁻¹	SOM g.kg ⁻¹	Al	Ca	Fe	K	Mg	Mn	Na	P	BD g.cm ⁻³	WC ₂₀₂₀ %	WC ₂₀₂₁ %
	cmol+.kg ⁻¹																				
A1	151	474	56	6.8	9	11	1.1	10	0.5	20	0.0	8	319	0.3	0.5	0.0	0.0	0.0	1.3	18	20
A2	210	381	119	6.3	12	19	1.8	11	0.5	34	0.0	11	290	0.6	0.8	0.1	0.0	0.0	1.3	22	16
A3	158	409	196	6.5	9	10	1.0	10	0.5	18	0.0	8	237	0.3	0.6	0.1	0.0	0.0	1.4	19	18
A4	426	237	93	7.9	31	20	2.3	9	20.7	34	0.0	30	244	0.9	1.3	0.0	0.1	0.0	1.1	25	25
A5	206	448	52	6.8	11	14	1.3	11	0.5	25	0.0	10	294	0.6	0.9	0.1	0.1	0.0	1.3	20	22
A6	277	378	29	6.6	16	12	1.3	10	0.5	21	0.1	14	316	0.4	1.0	0.0	0.1	0.0	1.3	18	19
A7	172	481	63	6.6	10	13	1.1	11	0.5	22	0.0	9	283	0.3	0.5	0.0	0.0	0.0	1.2	20	19
A8	193	364	183	5.6	8	14	1.3	11	0.5	24	0.3	6	261	0.5	0.7	0.2	0.0	0.0	1.5	19	15
A9	208	402	187	6.8	12	11	1.1	10	0.5	19	0.0	11	202	0.5	0.9	0.0	0.0	0.0	1.3	18	19
A10	220	442	47	7.2	13	12	1.2	10	0.7	21	0.0	11	292	0.6	0.9	0.0	0.1	0.0	1.3	18	18
A11	253	350	120	7.3	16	16	1.6	10	0.5	27	0.0	14	277	0.8	1.1	0.0	0.1	0.1	1.2	16	18
A12	221	352	164	7.4	14	14	1.4	10	2.2	23	0.0	13	262	0.7	0.7	0.0	0.0	0.0	1.3	18	16
A13	248	412	75	6.9	16	19	1.8	11	0.7	33	0.0	15	265	0.7	1.1	0.0	0.1	0.0	1.2	20	24
A14	175	385	120	6.8	11	19	1.7	11	0.5	32	0.0	11	320	0.3	0.5	0.0	0.0	0.0	1.2	18	20
A15	193	395	92	7.0	13	16	1.5	11	0.7	28	0.0	11	320	0.6	0.5	0.0	0.1	0.0	1.4	18	22
A16	284	338	67	8.1	22	14	1.4	10	23.3	24	0.0	22	311	0.4	0.5	0.0	0.1	0.0	1.4	20	19
A17	228	349	203	8.1	18	21	2.0	11	144.0	36	0.0	18	220	0.8	0.8	0.0	0.0	0.0	1.2	18	20
A18	196	455	68	6.8	11	14	1.2	11	0.5	24	0.0	10	281	0.2	0.7	0.0	0.0	0.0	1.2	20	20
A19	280	264	262	7.7	19	14	1.4	10	3.7	24	0.0	18	194	0.3	0.4	0.0	0.1	0.0	1.3	15	17
A20	204	407	49	7.5	12	10	1.0	10	0.5	17	0.0	11	340	0.2	0.6	0.0	0.1	0.0	1.4	17	14
A21	340	226	238	8.0	24	19	1.8	11	17.3	33	0.0	22	196	1.1	0.9	0.0	0.1	0.1	1.2	24	20

CEC: cation exchange capacity, SOC: soil organic carbon, TN: total nitrogen, SOM: soil organic matter, Al: aluminium, Ca: calcium, Fe: iron, K: potassium, Mg: magnesium, Mn: manganese, Na: sodium, P: phosphorus, BD: bulk density, WC: soil water content.

Table I. 6 Soil physico-chemical properties at 10-20 cm depth in the 21 plots of the study. Soil analyses were conducted in 2020, and water content was assessed in 2020 and 2021.

	Clay g.kg ⁻¹	Silt g.kg ⁻¹	Sand g.kg ⁻¹	pH	CEC cmol+.kg ⁻¹	SOC g.kg ⁻¹	TN g.kg ⁻¹	C/N	CaCO3 g.kg ⁻¹	SOM g.kg ⁻¹	Al	Ca	Fe	K	Mg	Mn	Na	P	BD g.cm ⁻³	WC ₂₀₂₀ %	WC ₂₀₂₁ %
	cmol+.kg ⁻¹																				
A1	158	475	53	7.1	9	9	0.9	10	0.5	15	0.0	9	314	0.2	0.3	0.0	0.0	0.0	1.4	17	19
A2	209	384	119	6.6	12	11	1.1	10	0.5	19	0.0	11	288	0.3	0.6	0.0	0.0	0.0	1.5	18	14
A3	156	418	191	6.5	9	11	1.1	10	0.5	19	0.0	8	234	0.3	0.6	0.1	0.0	0.0	1.5	18	18
A4	439	244	88	7.9	31	19	2.0	10	20.3	33	0.0	30	229	0.9	1.3	0.0	0.1	0.0	1.2	24	25
A5	214	448	51	6.2	11	11	1.1	10	0.5	19	0.1	10	287	0.4	0.8	0.1	0.1	0.0	1.5	18	19
A6	281	376	35	6.6	16	12	1.3	9	0.5	21	0.1	14	308	0.4	1.1	0.1	0.1	0.0	1.4	18	19
A7	175	426	62	6.7	10	14	1.2	11	0.5	23	0.0	9	337	0.4	0.6	0.0	0.1	0.0	1.3	18	19
A8	193	366	182	5.5	8	10	0.9	10	0.5	17	0.5	6	259	0.3	0.6	0.1	0.0	0.0	1.5	18	14
A9	203	406	188	6.8	12	11	1.1	10	0.5	18	0.0	11	203	0.4	0.9	0.0	0.1	0.0	1.4	18	18
A10	222	465	50	7.1	13	12	1.1	10	0.7	20	0.0	12	263	0.4	0.8	0.0	0.0	0.0	1.3	17	17
A11	259	352	113	7.5	16	17	1.6	10	0.7	29	0.0	14	276	1.1	1.2	0.0	0.1	0.1	1.2	17	17
A12	225	348	165	7.3	14	13	1.4	10	1.8	22	0.0	14	262	0.4	0.6	0.0	0.0	0.0	1.3	16	15
A13	252	411	71	6.9	16	16	1.5	10	0.5	27	0.0	14	265	0.4	0.9	0.0	0.1	0.0	1.4	17	21
A14	185	381	122	6.8	11	15	1.4	10	0.5	25	0.0	11	311	0.2	0.4	0.0	0.0	0.0	1.1	17	18
A15	195	392	89	6.6	12	14	1.3	10	0.5	24	0.0	10	324	0.3	0.5	0.0	0.0	0.0	1.5	19	20
A16	302	335	68	8.1	22	12	1.3	10	27.3	21	0.0	23	295	0.3	0.5	0.0	0.1	0.0	1.5	16	18
A17	228	351	192	8.2	18	18	1.7	11	143.3	31	0.0	17	229	0.4	0.7	0.0	0.0	0.0	1.4	16	18
A18	193	454	70	6.9	11	14	1.2	12	0.5	25	0.0	10	283	0.3	0.7	0.0	0.0	0.0	1.4	21	19
A19	280	267	260	7.7	19	16	1.6	10	4.2	28	0.0	19	192	0.4	0.5	0.0	0.1	0.0	1.3	17	16
A20	201	408	53	7.2	12	12	1.2	10	0.5	21	0.0	11	339	0.2	0.7	0.0	0.1	0.0	1.4	17	15
A21	333	225	249	7.9	23	16	1.7	10	17.0	28	0.0	22	194	0.6	0.8	0.0	0.1	0.0	1.3	21	21

CEC: cation exchange capacity, SOC: soil organic carbon, TN: total nitrogen, SOM: soil organic matter, Al: aluminium, Ca: calcium, Fe: iron, K: potassium, Mg: magnesium, Mn: manganese, Na: sodium, P: phosphorus, BD: bulk density, WC: soil water content.

The only significant differences in soil parameters between cropping systems are a higher content of K and Mg (ANOVA, $P < 0.05$) in OA than in Conv-CA systems at 10-20 cm depth (Table I.6). In addition, stable conservation systems tend to have a higher organic matter content at 0-10 cm than at 10-20 cm depth (31.30 and 22.41 g.kg⁻¹), and a higher bulk density than other systems, although these differences are not significant.

Practice intensity influenced soil properties, mostly at 10-20 cm depth (Table I.7). An increase in tillage intensity was related to a decrease in the C/N ratio at 0-10 cm depth, probably as organic matter is more homogeneously mixed in soil with tillage, thus decreasing the carbon content at the surface. At 10-20 cm depth, we observed an increase in soil K, Mg, Na and P contents as tillage intensity increased, a higher Mg content as pesticide treatment intensity decreased and a lower bulk density as fertilization intensity increased (Table I.7). Among primary practice indicators, an increase in the number of organic matter inputs (nbOrg) had a positive effect on numerous soil properties (CEC, SOC, TN, SOM, K, Mg, Na) at 10-20 cm depth (Table I.7).

Table I. 7 Effect of practice intensity on soil properties at 0-10 and 10-20 cm depth in 2020. Significant ($P < 0.05$) positive (+) or negative (-) effects were determined using a linear model.

	Composite indexes		Primary indicators	
	0-10 cm	10-20 cm	0-10 cm	10-20 cm
CEC			(+) nbOrg	(+) nbOrg
SOC				(+) nbOrg
TN				(+) nbOrg
C/N	(-) Itill		(+) soilP ; (-) nbSTill, nbOrg	(+) resExp
SOM				(+) nbOrg
Fe			(+) nbCrop	(+) nbCrop
K		(+) Itill		(+) nbOrg, nbDTill ; (-) soilP
Mg		(+) Itill ; (-) Itreat	(-) nbInsect	(+) nbOrg, nbDTill, nbSTill ; (-) soilP, nbInsect
Na		(+) Itill	(+) nbSTill	(+) nbOrg, nbSTill
P		(+) Itill	(-) soilP	(+) nbDTill ; (-) soilP
BD		(-) Ifertil		(-) resExp

Itill, Itreat, Ifertil: tillage, pesticide treatment and fertilization intensity index, nbCrop: nb of crops (5 y), nbDTill: nb of deep tillage, nbSTill: nb of surface tillage, soilP: type of tillage (5 y), nbInsect: nb of insecticides, nbOrg: nb of organic matter inputs (5 y), resExp: nb of residues exportations (5 y).

Note:

The format of the thesis, with chapters presented as articles, inevitably imply some redundancies. To avoid figure and table duplicates, when possible, we refer to this general introduction in the “materials and methods” part of the chapters.

———— Chapter 1 ————

Switching to indicators to unearth the effect of cropping systems on soil mesofauna

Juliette Chassain¹, Sophie Joimel¹, Antoine Gardarin², Laure Vieublé Gonod¹

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¹ Université Paris-Saclay, INRAE, AgroParisTech, UMR ECOSYS, 91120, Palaiseau, France

² Université Paris-Saclay, INRAE, AgroParisTech, UMR Agronomie, 91120, Palaiseau, France

Abstract

Soil organisms are impacted by a wide range of physical and chemical disturbances in intensive cropping systems. The development of cropping systems less disturbing to soil biodiversity requires to understand the consequences of various practices on soil organisms. However, most studies characterize the effect of cropping systems by distinguishing between the main types of cropping systems (i.e. conventional, organic, conservation) without taking into account the diversity of practices within those systems. In this study, we aim to better describe cropping systems and their effects on soil mesofauna using indicators of practice intensity developed by agronomists. Mesofauna sampling was conducted in autumn 2020 and 2021 over 21 plots under conventional, conservation or organic systems. Conservation or organic systems were either stable (≥ 7 years) or in transition (≤ 3 years). Simple indicators and composite indexes were computed to determine the intensity of tillage, pesticide treatments and fertilization. Mesofauna density and Collembola species and functional diversity were investigated using systems and intensity indexes as predictors. Collembola functional diversity was assessed with average species traits (i.e. trait from the literature) and by conducting length measurements directly on collected Collembola individuals (body, head, leg, antenna and furca length).

Mesofauna density and Collembola diversity tended to be the lowest in organic systems, whereas Collembola density was the highest in conservation systems, but the variability between years was high. High tillage intensity had a strong negative effect on mesofauna density and Collembola diversity in both years, whereas high pesticide treatment intensity had a positive effect but in 2020 only. In contrast, we found no effect of fertilization intensity. Species traits revealed additional effect of practice intensity on Collembola, with in particular a lower representation of traits showing adaptation to surface as tillage intensity increases (i.e. trichobothria, ocelli). Larger Collembola body length was observed under high pesticide treatment intensity in 2020 and under low tillage intensity in 2021. Other measured length traits revealed similar effects.

Overall, our results revealed that 1) practice intensity indexes can help to explain the effect of cropping systems on soil mesofauna, 2) taxonomic and functional diversity are complementary to understand the effects of practices on Collembola and 3) measurement of body length is relevant to assess effects of disturbances on the local Collembola community. However, soil mesofauna density and diversity were highly variable between plots and years. Future studies are thus necessary to fully assess the relevance of intensity indexes to explain the effect of cropping systems on soil biodiversity.

1.1 Introduction

The transition toward sustainable agroecosystems involves redesigning cropping systems by mobilizing agroecological principles, such as enhancing soil biodiversity and ecosystem functioning (Altieri, 1989; Wezel et al., 2009). Soil organisms, and more specifically mesofauna representatives, are key actors of agroecosystem functioning as they drive soil physical and chemical fertility (Crossley et al., 1992; Lavelle et al., 2006). Soil mesofauna includes numerous organisms ranging from 0.1 to 2 mm, mostly microarthropods, living in the first centimeters of soil (Swift et al., 1979; Lavelle and Spain, 2001). Collembola and Acari constitute a large part of soil microarthropods and play an essential role in soil organic matter decomposition (Moore et al., 1993; Chassain et al., 2021), nutrient dynamics (Mebes and Filser, 1998) and soil microstructure formation (Rusek, 1998). Consequently, they indirectly stimulate plant growth (Kreuzer et al., 2004). In addition, they are an important component of the soil food web, serving as prey for other arthropods (Hopkin, 1997) or consuming microorganisms and pest microfauna such as nematodes (Gerson, 2014; Potapov et al., 2022). Collembola in particular are considered to be good bioindicators of soil disturbances (Bispo et al., 2009; Socarrás and Izquierdo, 2014) and are a well-documented group in terms of species identification and functional traits in temperate areas (Potapov et al., 2020).

However, mesofauna density and diversity are lower in agroecosystems than in any other ecosystem (Santorufu et al., 2015; Joimel et al., 2017). In addition, we still lack understanding on the agronomic measures that could promote soil mesofauna as there is no clear consensus on the effect of cropping systems and practices on the different groups of soil organisms. Intensively cultivated soils were reported to have low density and species diversity of microarthropods (Winter et al., 1990; Rusek, 1998; Bedano et al., 2006). Intensive systems indeed rely on strong physical and chemical disturbances of soil, which may select organisms that are able to develop under changing conditions, namely species with a short life cycle, high reproduction capacity, and with a body size on average lower than in other ecosystem types (Kladivko, 2001; Cortet, 2010; Tsiafouli et al., 2015). Yet, it remains unclear if some practices are more disruptive than the others within these systems and how their interactions influence organism density and diversity.

Alternative systems, such as organic and conservation agriculture, have been developed to offset the drawbacks of intensive management. Organic farming is based on the ban of synthetic inputs (i.e. pesticides, mineral fertilizers), while conservation agriculture relies on no-tillage, permanent soil cover and diversification of the crop rotation. These alternatives were both reported to benefit soil mesofauna, in particular microarthropod density, compared to conventional systems. This was especially true for organic systems (Doles et al., 2001; Bettiol et al., 2002; Bengtsson et al., 2005). However, some studies reported no or negative effect of organic

farming on soil microarthropod density or richness (Schrader et al., 2006; Potapov et al., 2022). Comparatively, less studies were conducted on conservation agriculture (Christel et al., 2021). They found a positive effect on soil mesofauna abundance (Ayuke et al., 2019; Dulaurent et al., 2022). Similarly, reduced or no-tillage systems were reported to have a positive effect on microarthropod abundance and more variable results on diversity (Brennan et al., 2006; Tabaglio et al., 2009). Overall, higher density of several soil fauna groups was observed in conservation than in organic farming (Henneron et al., 2015), but more studies are required to compare the effect of alternative systems on the density of soil mesofauna (Christel et al., 2021). In addition, little is known about the effect of transition, from conventional to alternative systems or from one alternative to another, on mesofauna (Filser et al., 1995, 2002; Jabbour, 2008; Coller et al., 2022).

Previous studies also reported effects of single agricultural practices on soil mesofauna, in particular the impact of heavy tillage (Kladivko, 2001; Miyazawa et al., 2002), pesticides (Cortet et al., 2002; Miyazawa et al., 2002) and lack of organic inputs (Miyazawa et al., 2002; Reeleder et al., 2006). Tillage has been demonstrated to have important impacts on soil fauna, with in particular negative effects on microarthropods (Wardle, 1995; van Capelle et al., 2012; Moradi et al., 2013). In line with this, a recent meta-analysis reported a positive effect of reduced or no-tillage on mesofauna density (Betancur-Corredor et al., 2022). Negative effects of tillage could be explained by physical disturbances of soil causing direct (e.g. physical injuries) and indirect damage (e.g. destruction of microhabitats, drying of soil surface, changes in spatial distribution of the trophic resource) to microarthropods (Kladivko, 2001; Dang et al., 2015; Tsiafouli et al., 2015). However, other studies did not find any negative effect of tillage on soil mesofauna, and even positive effects on the abundance and richness of Collembola (van Capelle et al., 2012; Rieff et al., 2020). In addition, chemical disturbances due to pesticides can have direct (e.g. survival, reproduction) and indirect (e.g. decrease of feeding resource) effects on soil microarthropods, especially on Collembola (Cortet et al., 2002; Joimel et al., 2022). In contrast, the effect of fertilization on mesofauna depends on the type of fertilizers and seems more ambiguous. Organic fertilization tends to increase mesofauna density, whereas mineral fertilizers may have a neutral or negative effect (Miyazawa et al., 2002; Betancur-Corredor et al., 2023). However, when applied together, mineral and organic fertilizers can lead to a neutral effect of fertilization on Collembola (Kanal, 2004). Moreover, a reduced diversity of microarthropods was observed after manure input (Weil and Kroontje, 1979). Lastly, crop rotation and diversification were found to impact microarthropods density (Andrén and Lagerlöf, 1983; Jagers Op Akkerhuis et al., 2010).

Overall, the observed variability in mesofauna density and diversity is large in agricultural areas, probably due to the large panel of applied practices (Bengtsson et al., 2005). In most cases, studies on agricultural soils assess biodiversity by comparing fields assigned to the main cropping systems (i.e. conventional, integrated, organic, no-tillage or conservation) or considering only one

practice (e.g. tillage). The first approach conduces to a poor level of definition of practices applied to the field, while the second gives a partial description of the studied system. Indeed, tillage is often defined according to broad categories (conventional, reduced, no-tillage), thus merging practices without taking into account the parameters involved in the intensity of soil disturbances (e.g. depth or frequency of tillage). Similarly, pesticide treatments are often considered by comparing the presence/absence of treatments instead of considering separately products with different targets (e.g. herbicides, fungicides, insecticides) and application parameters (e.g. number, frequency and timing).

Consequently, agronomists highlighted the usefulness of an approach by indicators describing more finely the practices applied in cropping systems (Armengot et al., 2011; Bockstaller et al., 2015; Büchi et al., 2019). These indicators can be considered as a measure of practice intensity or as a measure of the related disturbances. They have the advantage to provide one value per plot instead of grouping plots into systems, thus switching from a discrete to a continuous value. In soil ecology, some authors already stated the interest of using indicators of the intensity and frequency of soil disturbances to compare the effect of different managements on soil organisms (Gareau et al., 2019; Masin et al., 2020). However, to our knowledge, this type of indicators was rarely used to assess the effect of cropping systems on soil organisms, more particularly for mesofauna. We found only one study using crop management descriptors to assess cropping system effect on microarthropods (Ferraro and Ghera, 2007). Here, we used indicators to assess the effect of physical and chemical disturbances on soil mesofauna and investigate whether they provide additional insight compared to a comparison between broad cropping systems.

The responses of soil organisms to disturbances, such as the ones caused by agricultural practices, are increasingly investigated using functional traits (Hedde et al., 2012; Pey et al., 2014; Salmon et al., 2014) with few studies focusing on Collembola (Santorufu et al., 2015; Martins da Silva et al., 2016; Maisto et al., 2017). The assessment of the functional traits within a community of soil invertebrates is often performed by attributing an average trait values to collected species (i.e. one trait value per species). These average species traits are extracted from database computing information from the literature. Fewer studies conducted traits measurements on individuals to assess effects of disturbances (Ribera et al., 2001; Fournier et al., 2012; Moretti et al., 2017). However, values of continuous traits (e.g. body length) may strongly vary between individuals of the same species due to local disturbances. Here, Collembola diversity was assessed using both taxonomic and functional traits approaches. Collembola traits were characterized both at the species level (i.e. average traits based on the literature) and individual level (i.e. measured body, head, leg, antenna and furca length on collected organisms).

In that context, the objectives of this study were 1) to assess if alternative cropping systems, stable or in transition, had beneficial effects on soil mesofauna, 2) to test whether the use of indexes of practice intensity (tillage, pesticide treatments, fertilization) bring new insight on those results, and 3) to characterize the effects of practice intensity on Collembola taxonomic and functional communities. We hypothesized that systems with lower physical (i.e. tillage) and chemical (i.e. pesticide treatments) disturbances, namely conservation and organic systems, will present higher density and diversity of mesofauna as well as a modified community composition compared to conventional systems. We assumed that these differences may vary between stable and transitional alternative systems and to mesofauna groups. We also expected that species traits will vary depending on the intensity of practices, with for instance larger Collembola species being the most sensitive to intensive tillage. Traits directly measured on individuals were presumed to provide more precise information on the influence of disturbances caused by practices on the Collembola community than species traits retrieved from the literature.

1.2 Materials and methods

1.2.1 Study sites

The study was conducted in autumn 2020 and 2021 over 21 plots owned by farmers located in the Paris basin, France (Table 1.1). Climate is temperate with mean annual precipitations of 600 to 700 mm and a mean annual temperature of 11°C. Total precipitations and min-max temperatures in October and November were 77-91 mm and 5-16°C in 2020, and 84-110 mm and 3-17°C in 2021 (Meteociel.fr). Meteorological conditions showed some differences in 2020 and 2021 (cf. General introduction).

All plots were cropped with winter wheat in 2020-2021 and with various crops or cover crop in 2021-2022 (Table 1.1). Soils were silty or clayed, with a pH ranging from 5.6 to 8.1 and a bulk density between 1.1 and 1.5 g.cm⁻³ at 0-10 cm depth (Table 1.1).

Plots were selected to represent six cropping systems. These systems were either i) established since seven to more than 20 years (i.e. stable) in conventional (Conv, n = 6 plots), organic (OA, n = 3) or conservation agriculture (CA, n = 3), or ii) in transition since 2 to 3 years from conventional to conservation (Conv-CA, n = 3), conventional to organic (Conv-OA, n = 3) or conservation to organic agriculture (CA-OA, n = 3) (Table 1.1).

Table 1. 1 Location, crop, cropping system and soil properties (0-10 cm depth) of the study plots. Age corresponds to the number of years since conversion to the current system in 2020.

Plot	Region	Crop 2020	Crop 2021	System	Age	Clay	Silt	Sand	SOM	pH	BD
						g.kg ⁻¹	g.kg ⁻¹	g.kg ⁻¹	g.kg ⁻¹		g.cm ⁻³
A1	Eure-et-Loir	wheat	wheat	Conv-CA	3	151.3	474.3	55.7	19.5	6.8	1.3
A2	Essonne	wheat	cover	CA	17	210.3	381.3	118.7	33.5	6.3	1.3
A3	Essonne	wheat	wheat	OA	20	157.7	409.0	196.3	17.5	6.5	1.4
A4	Yvelines	wheat	bare	OA	19	426.3	236.7	93.3	34.4	7.9	1.1
A5	Eure-et-Loir	wheat	barley	Conv	20	206.0	448.0	52.0	24.5	6.8	1.3
A6	Essonne	wheat	bare	CA-OA	2	277.3	377.7	28.7	21.0	6.6	1.3
A7	Yvelines	wheat	rye	Conv-OA	2	172.3	481.3	63.0	22.3	6.6	1.2
A8	Essonne	wheat	mustard	CA	7	192.7	363.7	182.7	24.0	5.6	1.5
A9	Yvelines	wheat	rapeseed	Conv	20	208.0	402.3	187.3	18.5	6.8	1.3
A10	Eure-et-Loir	wheat	alfalfa	Conv	20	219.7	441.7	46.7	20.9	7.2	1.3
A11	Yvelines	wheat	cover	OA	20	252.7	350.0	120.3	27.0	7.3	1.2
A12	Yvelines	wheat	cover	Conv	20	221.3	352.0	164.3	23.3	7.4	1.3
A13	Yvelines	wheat	rye, lentil	CA-OA	2	248.3	411.7	75.3	33.4	6.9	1.2
A14	Yvelines	wheat	cover	Conv-CA	3	175.0	385.0	119.7	32.0	6.8	1.2
A15	Yvelines	wheat	wheat	Conv-CA	3	193.3	395.3	91.7	28.1	7.0	1.4
A16	Essonne	wheat	mustard	Conv	20	284.3	338.0	66.7	24.2	8.1	1.4
A17	Yvelines	wheat	rapeseed	CA	10	228.0	349.0	203.3	36.4	8.1	1.2
A18	Yvelines	wheat	bare	Conv-OA	3	195.7	454.7	68.3	23.9	6.8	1.2
A19	Yvelines	wheat	clover	Conv-OA	2	279.7	264.0	262.0	23.8	7.7	1.3
A20	Eure-et-Loir	wheat	cover	CA-OA	2	204.0	407.0	49.3	16.9	7.5	1.4
A21	Eure-et-Loir	wheat	barley	Conv	20	340.0	226.3	238.0	32.9	8.0	1.2

Conv: conventional agriculture, CA: conservation agriculture, OA: organic agriculture, Conv-CA: transition from conventional to conservation (≤ 3 years), Conv-OA: transition from conventional to organic (≤ 3 years), CA-OA: transition from conservation to organic (≤ 3 years), SOM: soil organic matter, BD: bulk density.

1.2.2 Agricultural practices

Detailed information on the applied practices was collected by conducting a survey amongst the farmers. Indicators of practice intensity were selected to describe the cropping systems in each plot following Büchi et al. (2019). We decided to use indicators that were easy to obtain and that contributed to differentiate cropping systems. Selected indicators belonged to four categories: crop diversification, soil physical disturbances, chemical crop protection and fertilization (Table 1.2).

As our objective was to assess the effects of practices on soil organisms, we computed 2020 and 2021 indicators by considering all practices occurring during the previous crop cycle (i.e. from the soil preparation and sowing in autumn 2019 or 2020) and until the sampling date (i.e. beginning of new crop cycle in autumn 2020 or 2021). In addition, several indicators accounted for practices applied over the last five years, thus providing a mean to better account for the difference between stable and transitioning alternatives systems in our study.

Composite indexes were computed for tillage intensity (I_{till}) and fertilization intensity (I_{fertil}) by an additive combination of the primary indicators (Table 1.2) following Büchi et al., (2019). Primary indicators were first normalized to obtain values between 0 and 1:

$$I_{norm} = \frac{(I_i - I_{min})}{(I_{max} - I_{min})}$$

where I_{norm} is the normalized primary indicator, I_i is the indicator value at the plot i , I_{min} and I_{max} are the minimal and maximal indicator values within all plots. Then, composite indexes were calculated using the average of normalized indicators:

$$I_{composite} = \frac{\sum_{j=1}^n (I_{norm j})}{n}$$

where $I_{composite}$ is the composite intensity index, $I_{norm j}$ is the value of the normalized primary indicator j and n the number of indicators included in that index.

Regarding pesticides treatments, each product was considered as a separated treatment even when applied together with other products. We did not consider molluscicides as an individual treatment indicator as they were rarely applied. The composite index for pesticide treatment intensity (I_{treat}) was the normalized value of the total number of pesticide treatments applied to the plot (Table 1.2). For comparison, the treatment frequency index (TFI) (Jørgensen and Kudsk, 2006) was calculated over the crop cycle. However, as I_{treat} and TFI appeared to be highly correlated (Spearman correlation, $cor = 0.9$, $P < 0.001$ in 2020 and 2021) and as TFI was missing in two plots, we used only I_{treat} for the rest of the study.

Table 1. 2 Primary indicators and composite indexes of practice intensity used to characterize cropping systems. Composite indexes are given in bold. Time represents the time span in which data were collected to compute an indicator, either one year (1 y) from beginning of the previous cropping cycle until the sampling date, or five years (5 y) considered as crop rotation time. Indexes are adapted from Büchi et al. (2019).

Category	Name	Time	Content
(a) Crop diversification	nbCrop	5 y	Number of different crops in the last 5 years
(b) Soil physical disturbances (tillage)	nbDTill	1 y	Number of deep tillage (i.e. ploughing, > 15 cm depth)
	nbSTill	1 y	Number of surface tillage (i.e. all shallow tillage activities < 15 cm depth such as mechanical weeding, stubble incorporation, stones removal, seedbed preparation and rolling)
	soilP	5 y	Type of tillage in the last 5 years, calculated as the average of annual tillage weight (plough = 0.5, reduced tillage = 3, no-till = 5)
	Itill		Average of tillage indicators: (nbDTill + nbSTill + (1- soilP)) / 3
(c) Chemical crop protection (pesticide treatments)	nbHerb	1 y	Number of herbicide treatments
	nbFung	1 y	Number of fungicide treatments
	nbInsect	1 y	Number of insecticide treatments
	Itreat		Total number of pesticide treatments: herbicides, fungicides, insecticides, molluscicides and growth regulators
	TFI		Treatment frequency index comprising herbicides, fungicides, insecticides, molluscicides and seed treatments applied during the crop cycle (from sowing to harvest)
(d) Fertilization	nbOrg	5 y	Number of organic amendments in the last 5 years
	qminN	1 y	Quantity of mineral nitrogen fertilizers inputs (kg N.ha ⁻¹)
	resExp	5 y	Number of residues exportation in the last 5 years
	Ifertil		Average of fertilization indicators: (nbOrg + qminN + resExp) / 3

1.2.3 Soil sampling and physico-chemical analyses

Samplings were conducted from October 19th to December 2nd in 2020 and from October 25th to November 22nd in 2021. They took place minimum one week after sowing to allow the mesofauna population to partially recover after tillage and other mechanical operations related to sowing.

On each plot, three replicates were defined. They were spaced from each other by 25 m, located 25 m apart from the field margins and outside of tractor traffic tracks to avoid bias (Heisler and Kaiser, 1995; Reddersen, 1997). All samples were collected between crop rows.

Composite soil samples were collected at 0-10 cm depth on each replicate by mixing eight soil cores obtained with an auger. Before analyses, samples were kept at 4°C. Soil water content was assessed by drying fresh soil at 105°C for 48h. In 2020, a fraction of sampled soils was sieved at 4 mm, air dried and analyzed for main soil characteristics by the INRAE laboratory of Arras. Bulk density was assessed by collecting soil cores (10 cm in diameter, 10 cm high) at 0-10 cm depth, which were dried (105°C, 48h) and weighed. All soil parameters at the plot level are presented at 0-10 cm depth (cf. General introduction, Table I.5).

1.2.4 Mesofauna sampling

Mesofauna was sampled at 0-4 cm depth using small soil cores (5 cm in diameter, 4 cm high) on each replicate. Soil cores were kept at 4°C no longer than 8 days before extraction. Microarthropods were extracted using MacFayden extractor and following the NF/ISO 23611-2 norm. A gradient of temperature from 25°C to 45°C was progressively applied to the samples during the eight days of extraction.

Microarthropods were sorted under binocular into Collembola, Acari suborders and other mesofauna groups (e.g. small Myriapoda, Coleoptera and Diptera larvae, enchytraeids). Collembola were identified at the species level using phase-contrast microscope and identification keys (Bretfeld, 1999; Potapov, 2001; Hopkin, 2007). They were grouped into eco-morphological groups (euedaphic, hemiedaphic, epedaphic), which correspond to their vertical distribution in soil combined with morphological properties (Gisin, 1943). The taxonomic diversity of Collembola was estimated by calculating the number of species, the Shannon index and the Pielou's evenness index. We calculated the ratio Acari/Collembola as a measure of disturbance. A low ratio is assumed to be related to high disturbances as Acari are considered to be less tolerant than Collembola (Bachelier, 1963; Joimel et al., 2017).

1.2.5 Collembola functional traits assessment

Collembola species traits were selected in order to understand species responses to disturbances caused by cropping practices. We selected eight morphological traits (trait attributes are presented in parenthesis): body length (< 1.5 mm, > 1.5 mm), body shape (cylindrical, spherical), furca type (long, short/absent), number of ocelli (0, 1-5, ≥ 6), and presence/absence of post-antennal organ (i.e. PAO), pigmentation, scales and trichobothria. These traits were used as they could be related to distribution in soil and dispersal abilities (Vandewalle et al., 2010). We also took into account the reproduction type (sexual, parthenogenetic) and ability for ecomorphosis (presence, absence) as they could be good indicators of the recovery or resistance facing disturbances. Indeed, the reproduction type represents the ability of Collembola to recolonize an area after disturbances (e.g. tillage) as parthenogenetic populations have a higher ability to recover after being disturbed. The motion strategy was represented by the furca presence/absence and size, which determine the ability to jump and to escape predators. All traits for Collembola species were collected from the BETSI database (CESAB/FRB) containing data extracted from the literature (Pey et al., 2014). Using species traits, we calculated the functional richness (FRic), which represents the volume of the functional space occupied by the community, and the functional evenness (FEve) which corresponds to the regularity of the distribution of species density in this volume (Villéger et al., 2008).

Additionally, length traits were measured on collected Collembola individuals to account for actual traits of the local community. Length of the body, head, leg, antenna and furca were measured using Flexacam C1 camera on DM2000 microscope (Leica) and the Leica Application Suite X software. Up to 10 individuals per species and per sample were measured. Lengths were measured along the main axis of each element, taking into account inflexion points (Figure 1.1).

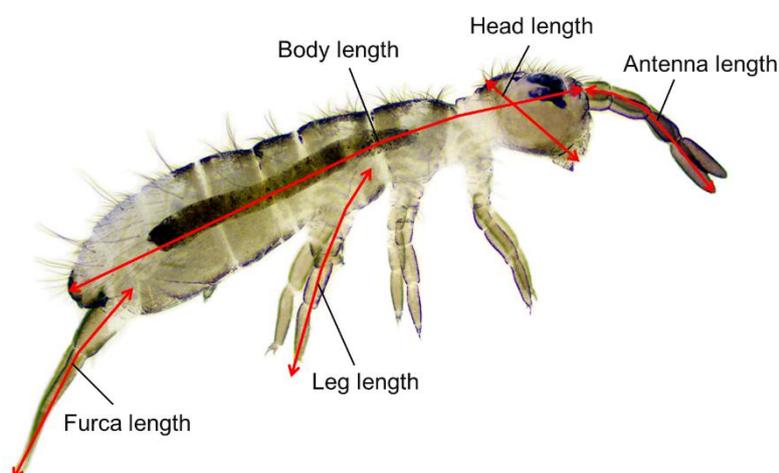


Figure 1. 1 Measurement of length traits on Collembola. Red arrows represent the position of the different length measurements.

Two measurements were performed for the body length: 1) total length from the posterior end of the abdomen to the head between the antennas, 2) headless length from the posterior end of the abdomen to the anterior end of the first thoracic segment. As both measurements were highly correlated (Spearman correlation, $cor = 0.99$, $P < 0.001$ in 2020 and 2021), we decided to keep the total length including head, hereafter called body length. Head length is the maximum diagonal length from top of head to the labrum. Leg length is the average length of both posterior legs (i.e. third pair of legs) from claw tip to top part of coxae. Antenna length is to the length of all antenna segments. Lastly, furca length comprises the length of manubrium, dens and mucro.

1.2.6 Statistical analyses

Prior to statistical analyses, the three replicates were averaged to obtain one value per plot. Densities were obtained by dividing abundance by the area of soil extraction (0.0025 m^2). The normality and homogeneity of variances were tested with a Shapiro-Wilk test ($\alpha > 0.05$) and a Bartlett test ($\alpha > 0.05$).

A principal component analysis (PCA) was performed on primary practice indicators to assess their ability to distinguish between the different cropping systems. Differences in practice intensity between cropping systems were assessed by comparing composite indexes with an analysis of variance (ANOVA) followed by a Tukey HSD post-hoc test ($P < 0.05$).

As normality and homogeneity of variance were not met, the relations between cropping systems or practice intensity and soil mesofauna density and diversity parameters were assessed using generalized linear models (GLM) with a quasi-Poisson family and an identity link (i.e. accounting for overdispersion of data), followed by Tukey HSD post-hoc tests when comparing systems. In addition, GLM models were computed to assess the interaction effects of the three intensity indexes. However, no interaction effect was recovered for the three composite indexes regarding soil mesofauna (Table S1.1). Hence, only the simplest models, looking at each intensity index separately, are presented in the following parts.

The effects of cropping systems and years on Collembola community composition were assessed by performing a principal coordinates analysis (PCoA) using Bray-Curtis distance, which allowed looking at the dissimilarities between communities. It was followed by a permutational analysis of similarities (ANOSIM) to test for significant differences between systems and years.

For each species trait, the community-weighted mean (CWM) was calculated as the average trait values weighted by the density of species in the community (Lavorel et al., 2008). A PCA was performed on CWM to assess the functional composition of Collembola communities. Responses of trait attributes to cropping systems and practice intensity were assessed with similar analyses than for mesofauna density and diversity (i.e. GLM, Tukey post-hoc tests).

For measured individual traits, sub-samples were not pooled in order to consider each *Collembola* individual as a data point. Relations between cropping systems or practice intensity and traits were investigated using generalized linear mixed-effect models (GLMM) with a negative binomial distribution, fixed effect for system or intensity index and random effect for plots, followed by Tukey HSD post-hoc tests when comparing different systems.

All the analyses were performed using R software version 3.6.3 (R Development Core Team 2020) and the *stats* (R Development Core Team 2020), *ade4* (Dray and Dufour, 2007), *vegan* (Oksanen et al., 2022), *FD* (Laliberté et al., 2022), *lme4* (Bates et al., 2015) and *multcomp* (Hothorn et al., 2022) packages.

1.3. Results

1.3.1 Primary indicators and intensity indexes

Values of primary indicators and composite indexes are presented for each system (Table 1.3) and for each plot (cf. General introduction, Table I.4). As shown by the PCA, primary indicators enabled to differentiate systems in 2020 (total inertia: axis 1 = 41.6%, axis 2 = 19.0%) and in 2021 (axis 1 = 47.7%, axis 2 = 15.4%). Organic systems were clearly separated from conventional and conservation systems both of which overlapped (cf. General introduction, Figure I.9).

Composite indexes I_{till} and I_{treat} were significantly different between systems, but not I_{fertil} (Table 1.3). Unsurprisingly, we observed a significantly lower tillage intensity in conservation systems and an absence of pesticide treatments in organic systems (Table 1.3). Conversely, we found no significant difference between conventional and organic systems for tillage intensity nor between conventional and conservation systems for pesticide treatment intensity (Table 1.3). However, tillage intensity was higher in most organic plots than in conventional plots in 2020, except for one CA-OA plot (A13) and one Conv plot (A9) (Figure 1.2a). In 2021, tillage intensity was high for all plots of stable organic systems (Figure 1.2d). The I_{treat} index varied over a large range of values for conventional and conservation systems, especially in 2020 (Figures 1.2b and e). One CA plot (A8) had a pesticide treatment intensity almost twice as small as that of other conservation plots. In contrast, the I_{fertil} index illustrated that fertilization intensity was not system dependent (Table 1.3, Figures 1.2c and f). However, primary indicators showed that long term organic matter input was significantly higher in stable organic systems in both years, and that mineral nitrogen input was higher in conventional than in stable conservation systems in 2021 (Table 1.3). Regarding transitioning systems, intensity indexes were similar between Conv and Conv-CA, whereas I_{treat} was higher in Conv than in Conv-OA and in CA than in CA-OA in both years (Table 1.3). In addition, I_{till} was lower in CA than in CA-OA in 2020 (Table 1.3).

Table 1. 3 Values of primary indicators and composite indexes of practice intensity in studied cropping systems in 2020 and 2021. Values are means \pm standard deviations. Normalized values are presented for Itill, Itreat and Ifertil (rows in grey). Different lower-case letters and bold values indicate significant differences between systems (ANOVA, $P < 0.05$).

	2020						2021					
	Conv	CA	Conv-CA	OA	Conv-OA	CA-OA	Conv	CA	Conv-CA	OA	Conv-OA	CA-OA
nbCrop	3.2 \pm 0.8	3.3 \pm 0.6	3.7 \pm 0.6	3.7 \pm 0.6	3.0 \pm 1.0	3.3 \pm 0.6	3.5 \pm 1.1	3.7 \pm 0.6	3.7 \pm 0.6	3.7 \pm 0.6	3.7 \pm 1.2	3.7 \pm 0.6
nbDTill	0.7 \pm 0.8	0	0	1.7 \pm 0.6	1.7 \pm 0.6	1.0 \pm 1.0	0.7 \pm 0.8	0	0	1.3 \pm 0.6	1.3 \pm 0.6	0.3 \pm 0.6
nbSTill	5.3 \pm 3.1	0.7 \pm 1.2	0.3 \pm 0.6	6.3 \pm 2.5	3.7 \pm 1.2	7.0 \pm 4.6	4.0 \pm 2.5	0	0.3 \pm 0.6	7.7 \pm 1.2	7.0 \pm 2.7	8.3 \pm 5.9
soilP	2.0 \pm 1.0	4.9 \pm 0.2	3.8 \pm 0.4	1.3 \pm 1.3	1.5 \pm 1.0	2.3 \pm 0.8	2.0 \pm 1.1	4.9 \pm 0.2	4.1 \pm 0.6	1.3 \pm 1.3	1.5 \pm 1.0	2.3 \pm 0.8
Itill	0.5 \pm 0.2	0.0 \pm 0.1	0.1 \pm 0.0	0.7 \pm 0.3	0.6 \pm 0.1	0.6 \pm 0.2	0.4 \pm 0.2	0	0.1 \pm 0.1	0.7 \pm 0.1	0.6 \pm 0.2	0.4 \pm 0.1
nbHerb	5.2 \pm 1.9	6.3 \pm 4.0	5.3 \pm 0.6	0	0	0	7.8 \pm 1.2	6.0 \pm 2.7	6.3 \pm 3.5	0	0	0
nbFung	0.8 \pm 0.4	0.3 \pm 0.6	1.0 \pm 1.0	0	0	0	1.8 \pm 0.4	1.7 \pm 1.5	3.3 \pm 0.6	0	0	0
nbInsect	1.3 \pm 1.0	0	2.3 \pm 0.6	0	0	0	0.8 \pm 0.8	0.7 \pm 1.2	2.0 \pm 2.0	0	0	0
Itreat	0.7 \pm 0.2	0.6 \pm 0.4	0.8 \pm 0.0	0	0	0	0.8 \pm 0.1	0.6 \pm 0.3	0.8 \pm 0.3	0	0	0
TFI	5.0 \pm 1.4	3.1 \pm 1.9	6.4 \pm 0.0	0	0	0	9.4 \pm 1.6	5.9 \pm 4.2	6.6 \pm 0.0	0	0	0.3 \pm 0.5
nbOrg	0.8 \pm 1.0	0.7 \pm 0.6	1.0 \pm 1.0	5.0 \pm 1.7	3.3 \pm 0.6	2.3 \pm 0.6	0.8 \pm 1.0	0.7 \pm 0.6	1.0 \pm 1.0	5.0 \pm 1.7	3.3 \pm 0.6	3.0 \pm 1.0
qminN	129 \pm 67	109 \pm 95	69 \pm 70	0	0	0	198 \pm 41	130 \pm 40	187 \pm 24	0	0	0
resExp	0.3 \pm 0.8	0	2.3 \pm 2.1	1.0 \pm 1.7	2.3 \pm 1.2	0	0.3 \pm 0.8	0	2.3 \pm 2.1	1.0 \pm 1.7	2.3 \pm 1.2	0.3 \pm 0.6
Ifertil	0.3 \pm 0.2	0.2 \pm 0.2	0.4 \pm 0.1	0.4 \pm 0.2	0.4 \pm 0.1	0.1 \pm 0.0	0.3 \pm 0.1	0.2 \pm 0.0	0.5 \pm 0.2	0.4 \pm 0.2	0.4 \pm 0.1	0.2 \pm 0.0

Conv, CA and OA: conventional, conservation and organic agriculture, Conv-CA, Conv-OA and CA-OA: initial-recent system transitions, nbCrop: nb of crops (5 y), nbDTill: nb of deep tillage, nbSTill: nb of surface tillage, soilP: type of tillage (5 y), Itill: tillage intensity index, nbHerb: nb of herbicides, nbFung: nb of fungicides, nbInsect: nb of insecticides, Itreat: pesticide treatment intensity index, TFI: treatment frequency index, nbOrg: nb of organic matter inputs (5 y), qminN: quantity of mineral nitrogen (kg N.ha⁻¹), resExp: nb of residues exportations (5 y), Ifertil: fertilization intensity index.

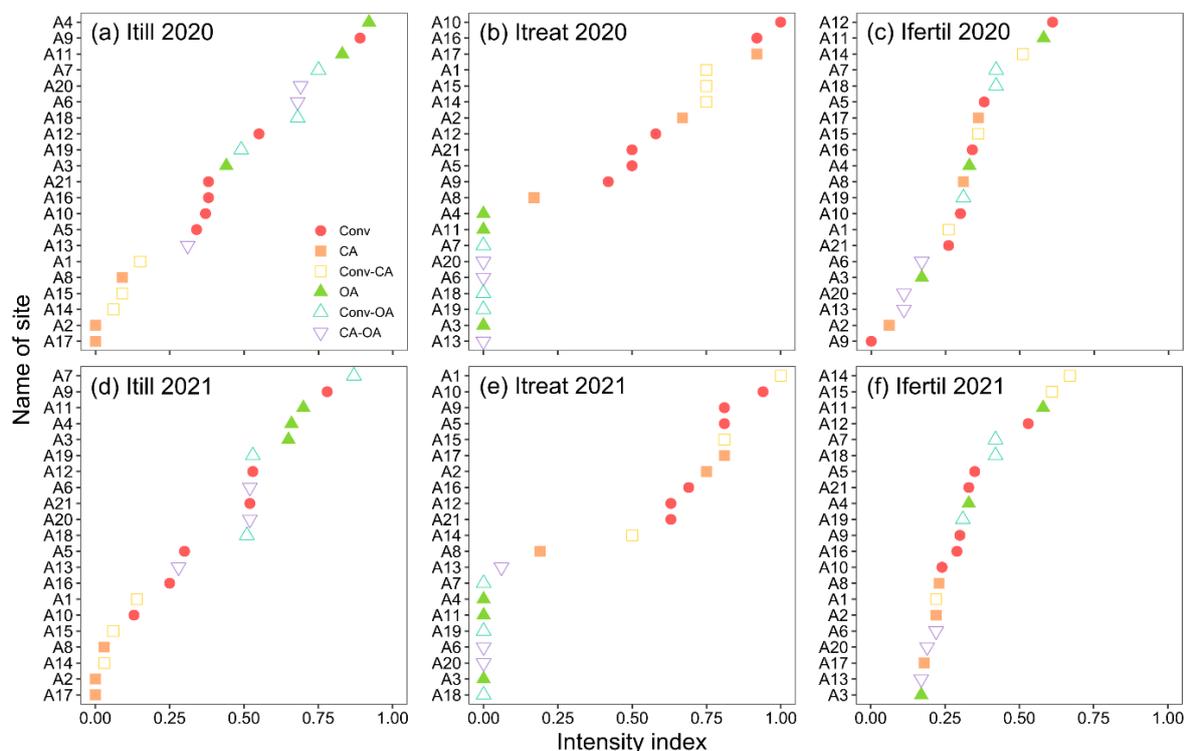


Figure 1. 2 Composite indexes for tillage (Itill), pesticide treatments (Itreat) and fertilization (Ifertil) intensity in all plots in 2020 and 2021. Symbols correspond to system groups as reported in the legend. Conv: conventional agriculture; CA: conservation agriculture; OA: organic agriculture; Conv-CA, Conv-OA and CA-OA: initial-recent system transitions.

1.3.2 Effect of cropping systems and practice intensity on mesofauna density

Mesofauna density was on average lower in 2020 (20 462 ind.m⁻²) than in 2021 (32 249 ind.m⁻²) with a large variability between plots (Figure 1.3). This trend was observed in all systems except conventional system due to a lower Acari density in 2021. Very few organisms were collected in organic systems in 2020, in particular in Conv-OA (Figure 1.3). However, mesofauna variables were not significantly influenced by the soil water content (GLM, $P > 0.05$) nor by the different crops in 2021 (GLM, $P > 0.05$).

Total mesofauna and Acari (total, Oribatida, Gamasida) densities were significantly lower in OA and Conv-OA than in Conv in 2020, but we observed no significant difference between systems in 2021 (Figure 1.3, Table 1.4). Collembola density was significantly higher in Conv and Conv-CA than in Conv-OA in 2020, and in CA than in CA-OA systems in 2021 (Figure 1.3) corresponding to effects observed on hemiedaphic species (Table 1.4a). Euedaphic species density was significantly higher in Conv than in Conv-OA in 2020, and in OA than in CA-OA in 2021 (Table 1.4a). Epedaphic density did not differ significantly between systems. The ratio Acari/Collembola was significantly higher in CA-OA than in other systems, except Conv-OA, in 2021 (Table 1.4d).

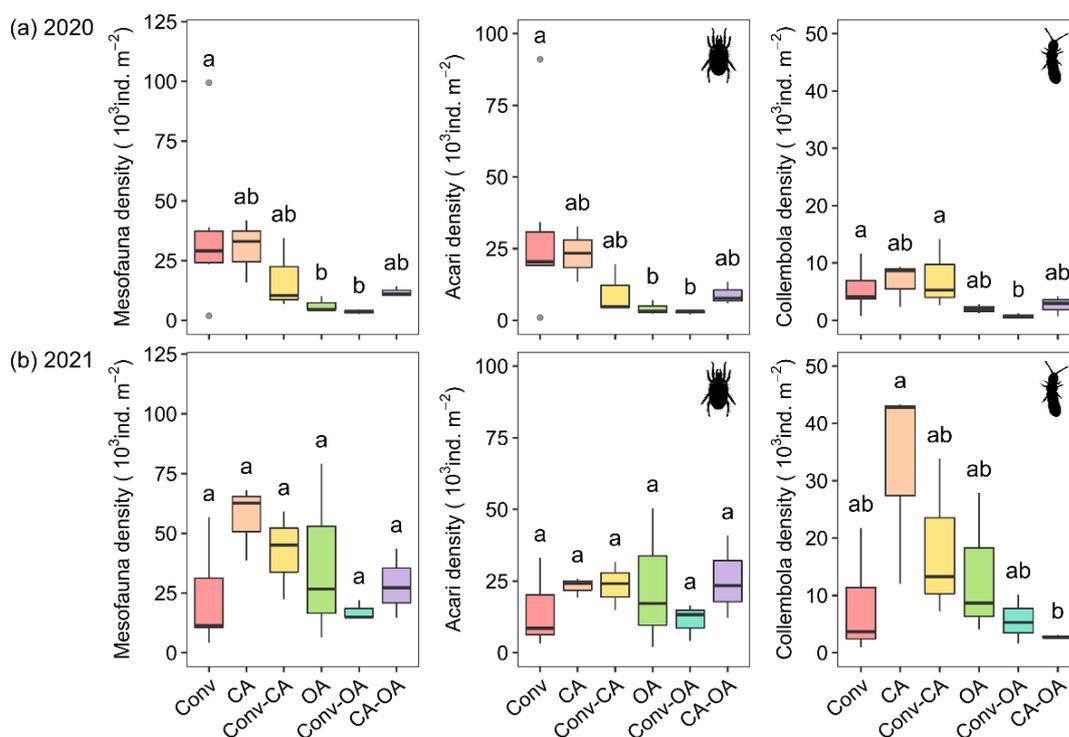


Figure 1. 3 Density of total mesofauna, Acari and Collembola in studied cropping systems in (a) 2020 and (b) 2021. Different lower-case letters indicate significant differences between cropping systems according to the results of GLM with a quasi-Poisson error distribution ($P < 0.05$). Conv: conventional agriculture, CA: conservation agriculture, OA: organic agriculture, Conv-CA, Conv-OA and CA-OA: initial-recent system transitions.

Table 1. 4 Density (10^2 ind.m⁻²) of (a) Acari orders, (b) Collembola ecomorphological groups and (c) other mesofauna representatives, and (d) ratio Acari/Collembola in studied cropping systems in 2020 and 2021. Values are means \pm standard deviations. Different lower-case letters and bold values indicate significant differences between cropping systems according to the results of GLM with a quasi-Poisson error distribution ($P < 0.05$).

	2020						2021					
	Conv	CA	Conv-CA	OA	Conv-OA	CA-OA	Conv	CA	Conv-CA	OA	Conv-OA	CA-OA
(a) Acari												
Actinidida	1 \pm 2	6 \pm 7	13 \pm 23	1 \pm 0	0 \pm 1	7 \pm 3	4 \pm 5	0 \pm 1	6 \pm 11	0 \pm 1	0 \pm 1	9 \pm 8
Gamasida	28 \pm 17 a	34 \pm 24 ab	15 \pm 12 ab	10 \pm 7 ab	5 \pm 2 b	9 \pm 8 ab	22 \pm 13	34 \pm 10	22 \pm 11	44 \pm 47	18 \pm 12	32 \pm 11
Oribatida	280 \pm 305 a	192 \pm 91 ab	67 \pm 56 ab	30 \pm 28 b	24 \pm 8 b	73 \pm 48 ab	113 \pm 109	197 \pm 43	207 \pm 95	188 \pm 201	94 \pm 61	214 \pm 126
(b) Collembola												
Epedaphic	88 \pm 97	134 \pm 86	149 \pm 171	31 \pm 5	16 \pm 15	55 \pm 47	45 \pm 58	157 \pm 117	96 \pm 50	30 \pm 13	28 \pm 15	18 \pm 3
Euedaphic	25 \pm 25 a	9 \pm 8 ab	11 \pm 12 ab	4 \pm 4 ab	1 \pm 2 b	8 \pm 7 ab	3 \pm 5 ab	4 \pm 4 ab	8 \pm 9 ab	21 \pm 20 a	2 \pm 2 ab	0 \pm 0 b
Hemiedaphic	48 \pm 33 a	60 \pm 37 a	62 \pm 21 a	24 \pm 23 ab	3 \pm 2 b	15 \pm 5 ab	28 \pm 26 ab	166 \pm 104 a	78 \pm 80 ab	84 \pm 94 ab	28 \pm 30 ab	9 \pm 3 b
(c) Other	6 \pm 5 a	3 \pm 1 ab	4 \pm 3 ab	2 \pm 1 ab	0 \pm 1 b	2 \pm 1 ab	4 \pm 7	6 \pm 2	6 \pm 6	6 \pm 3	3 \pm 3	2 \pm 4
(d) Ratio A/C	5.5 \pm 4.0	4.0 \pm 1.7	1.3 \pm 0.3	2.1 \pm 0.8	6.2 \pm 5.1	7.9 \pm 10.3	2.4 \pm 1.0 b	1.1 \pm 0.9 b	1.7 \pm 0.9 b	1.4 \pm 0.8 b	3.9 \pm 3.9 ab	9.2 \pm 5.3 a

Conv, CA and OA: conventional, conservation and organic agriculture; Conv-CA, Conv-OA and CA-OA: initial-recent system transitions; Ratio A/C: ratio Acari/Collembola.

Tillage and pesticide treatment intensities demonstrated significant effects on soil mesofauna density, while we found no clear relation with fertilization intensity (Table 1.5). An increase in tillage intensity was related to a decrease in total mesofauna and Collembola densities in both years (Table 1.5a and b) and of Acari (total, Gamasida, Oribatida) in 2020 only (Table 1.5a). Inversely, all mesofauna groups showed a significantly higher density as pesticide treatment intensity increased in 2020 (Table 1.5a) but not in 2021 (Table 1.5b). Epedaphic Collembola represented the only group for which density was significantly impacted by tillage and pesticide treatment intensities in both years (Table 1.5a and b). Actinidida density was negatively impacted by fertilization intensity in 2020 (Table 1.5a).

Primary practice indicators provided additional information on the effect of cropping systems on mesofauna density (Table 1.5). All the indicators influenced mesofauna density but their effects varied among groups and years.

Table 1. 5 Effect of practice intensity on the density of mesofauna groups in (a) 2020 and (b) 2021. *t*-value and *P*-value were obtained using GLM with a quasi-Poisson error distribution. Bold values indicate significant effects ($P < 0.05$ *, < 0.01 **, < 0.001 ***). Only primary indicators having a significant ($P < 0.05$) positive (+) or negative (-) effect on density are reported.

	Itill		Itreat		Ifertil		Indicators
	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	
(a) 2020							
Total mesofauna	-2.64	0.016*	2.92	0.009**	0.57	0.576	(+) nbHerb, qminN ; (-) nbDTill, nbOrg
Acari	-2.28	0.035*	2.38	0.028*	0.64	0.531	(+) nbHerb, qminN ; (-) nbDTill, nbOrg
Actinidida	-1.81	0.086	0.86	0.401	-2.46	0.024*	(-) nbFung
Gamasida	-3.99	<0.001***	3.84	0.001**	1.08	0.295	(+) soilP, nbHerb, qminN ; (-) nbDTill, nbOrg, resExp
Oribatida	-1.96	0.065	2.15	0.044*	0.64	0.529	(+) nbHerb, qminN ; (-) nbDTill
Collembola	-3.64	0.002**	4.55	<0.001***	-0.17	0.868	(+) soilP, nbHerb, qminN ; (-) nbDTill, nbSTill, nbOrg
Epedaphic	-3.36	0.003**	3.27	0.004**	-1.02	0.319	(+) soilP, nbHerb, qminN ; (-) nbDTill, nbSTill, nbOrg, resExp
Euedaphic	-0.31	0.759	2.29	0.034*	0.84	0.414	(+) nbInsect, qminN
Hemiedaphic	-2.97	0.008**	4.00	<0.001***	0.69	0.498	(+) soilP, nbHerb ; (-) nbDTill
Other	-1.01	0.325	3.53	0.002**	0.70	0.491	(+) nbHerb, nbInsect, qminN
(b) 2021							
Total mesofauna	-2.23	0.038*	0.57	0.572	-0.94	0.361	(+) nbCrop ; (-) nbDTill
Acari	-1.28	0.218	-0.13	0.901	-0.48	0.638	(+) nbCrop ; (-) nbDTill
Actinidida	-0.52	0.607	0.76	0.455	-0.11	0.911	(-) nbInsect
Gamasida	-0.19	0.848	-1.05	0.308	-0.92	0.368	n.s.
Oribatida	-1.38	0.182	-0.03	0.974	-0.32	0.750	(+) nbCrop ; (-) nbDTill
Collembola	-2.81	0.011*	1.10	0.287	-1.16	0.259	(-) nbDTill, nbSTill
Epedaphic	-4.46	<0.001***	2.22	0.039*	-0.91	0.372	(+) soilP, nbHerb, nbCrop ; (-) nbDTill, nbSTill
Euedaphic	0.57	0.577	-0.50	0.623	-0.04	0.972	n.s.
Hemiedaphic	-1.60	0.125	0.36	0.723	-1.26	0.222	(-) nbSTill
Other	-1.37	0.187	1.19	0.250	-1.16	0.260	(-) nbSTill

nbCrop: nb of crops (5 y), nbDTill: nb of deep tillage, nbSTill: nb of surface tillage, soilP: type of tillage (5 y), Itill: tillage intensity index, nbHerb: nb of herbicides, nbFung: nb of fungicides, nbInsect: nb of insecticides, Itreat: pesticide treatment intensity index, TFI: treatment frequency index, nbOrg: nb of organic matter inputs (5 y), qminN: quantity of mineral nitrogen (kg N.ha⁻¹), resExp: nb of residues exportations (5 y), Ifertil: fertilization intensity index, n.s. : no significant effect.

1.3.3 Effect of cropping systems and practice intensity on Collembola taxonomic diversity

A total of 30 and 43 Collembola species were collected in 2020 and 2021 respectively. Collembola species richness was significantly higher in Conv, CA and Conv-CA than in Conv-OA in 2020, but we observed no differences between systems in 2021 (Figure 1.4a). In addition, species richness significantly decreased with the increase in tillage intensity in 2020, with similar trend in 2021, and increased as pesticide treatment intensity increased in 2020 only (Figure 1.4a). Shannon index followed similar trends as species richness. Pielou's evenness index had a negative response to increased pesticide treatment intensity in 2020 (GLM, $P = 0.025$). All diversity values are reported at the plot level in Table S1.2.

The community composition of Collembola was significantly different in 2020 and 2021 (ANOSIM: $R = 0.130$, $P = 0.005$) (Figure S1.1). Conventional and conservation systems had similar communities, differing significantly from communities in organic systems in 2020 (ANOSIM system: $R = 0.241$, $P = 0.021$), but not in 2021 (ANOSIM system: $R = 0.078$, $P = 0.231$) (Figure S1.1). Differences were related to the absence or low occurrence of several species in organic systems, but we did not find species that are representatives of the different cropping systems (Appendix

1.1, Table S1.3). Collembola species which were significantly influenced by practice intensity all had a negative response to tillage intensity and a positive response to pesticide treatment intensity (Table S1.4). In particular, significantly higher densities of *Lepidocyrtus cyaneus* Tullberg, 1871 and *Pseudosinella alba* (Packard, 1873) were observed under low tillage and high pesticide treatment intensities in both years.

1.3.4 Effect of cropping systems and practice intensity on Collembola species traits

Collembola functional richness was on average similar in 2020 and in 2021. It was significantly lower in Conv-OA than in Conv-CA in 2020, but we found no difference between systems in 2021 (Figure 1.4b). In addition, functional richness was negatively related to tillage intensity in both years, positively related to pesticide treatment intensity in 2020 and not influenced by fertilization intensity (Figure 1.4b). Functional evenness was not significantly different between systems (Table S1.5).

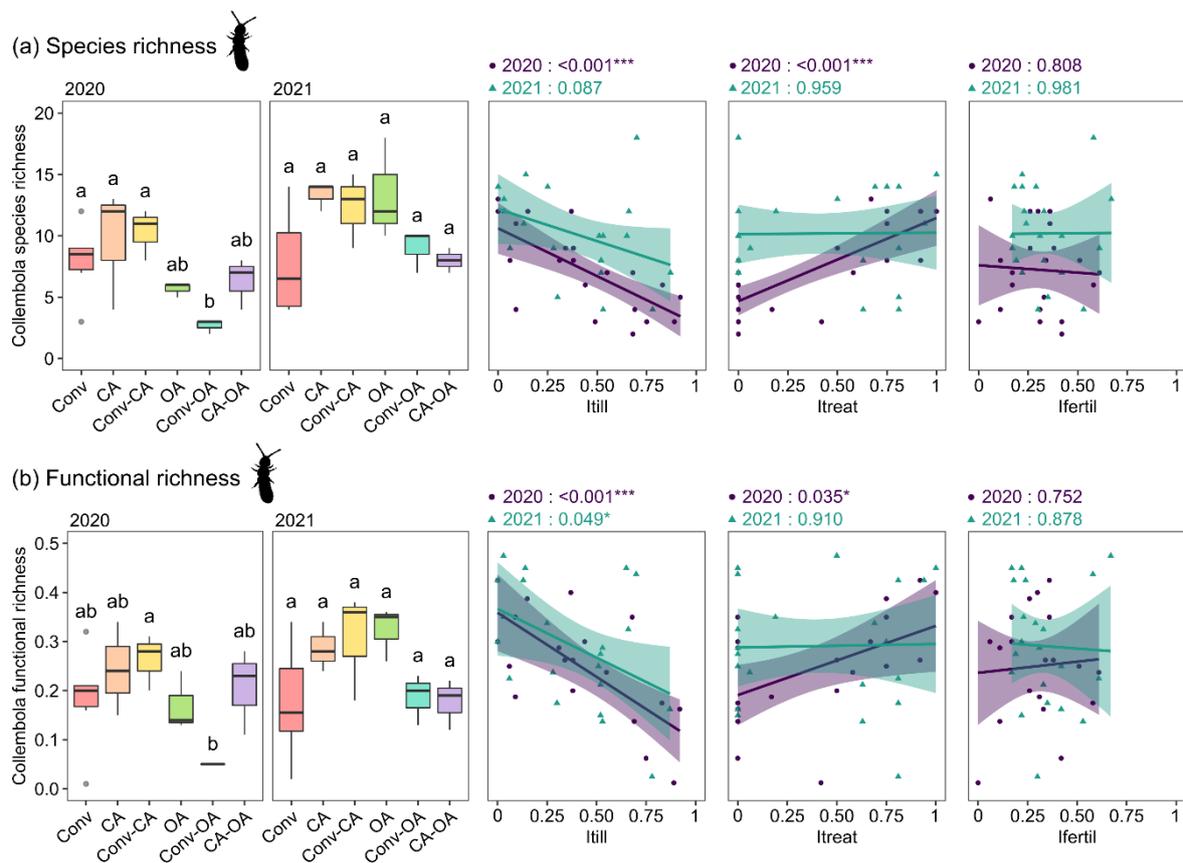


Figure 1. 4 Effect of cropping systems and practice intensity on Collembola (a) species richness and (b) functional richness in 2020 and 2021. Significant difference between systems is represented with different lower-case letters and significant effects of intensity indexes are reported for each year ($P < 0.05$ *, < 0.01 **, < 0.001 ***), all according to the results of GLM with a quasi-Poisson error distribution. Conv, CA and OA: conventional, conservation and organic agriculture; Conv-CA, Conv-OA and CA-OA: initial-recent system transitions, ltill: tillage intensity, ltreat: pesticide treatment intensity, lfertil: fertilization intensity.

Functional communities did not differ significantly between systems according to PCA based on CWM of Collembola species traits (Figure S1.2). In addition, in 2020 we found no significant difference in traits CWM between systems, while in 2021 we observed a higher representation of individuals without ocelli in OA and with cylindrical body shape in CA and Conv-CA compared to CA-OA (Table S1.5).

As tillage intensity increased, we observed a lower representation of Collembola species with trichobothria in 2020 and similar trend for scales in 2021. Inversely, we found a higher representation of Collembola without ocelli in both years and that tended to have a smaller body length, PAO or parthenogenetic reproduction type depending on years (Table 1.6). An increase in pesticide treatment intensity was significantly related to a higher representation of Collembola with scales in 2020, with a similar trend in 2021 (Table 1.6).

Table 1. 6 Effect of practice intensity on Collembola species traits in 2020 and 2021. *t*-value and *P*-value were obtained using GLM with a quasi-Poisson error distribution. Bold values indicate significant effect ($P < 0.05$ *, < 0.01 **, < 0.001 ***). Only attributes of species traits showing a significant or strong relation to at least one practice are reported.

	2020						2021					
	Itill		Itreat		Ifertil		Itill		Itreat		Ifertil	
	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>
BL<1.5	0.21	0.839	-1.32	0.203	-0.67	0.510	1.85	0.080	-1.02	0.319	-0.71	0.485
Oce_0	1.78	0.091	0.30	0.764	-0.57	0.577	2.37	0.028*	-1.63	0.119	0.51	0.615
SCA_present	-0.86	0.400	2.42	0.026*	0.28	0.782	-1.75	0.096	1.77	0.092	0.17	0.868
PAO_present	1.88	0.075	-1.00	0.332	0.37	0.715	0.66	0.516	-0.14	0.892	-0.10	0.921
TRI_present	-2.11	0.049*	0.55	0.588	-0.61	0.552	-1.12	0.277	0.28	0.787	0.38	0.710
REP_parth	1.61	0.123	0.04	0.969	0.42	0.678	1.77	0.092	0.39	0.703	0.19	0.850

Itill: tillage intensity index, Itreat: pesticide treatment intensity index, Ifertil: fertilization intensity index, BL<1.5: body length of less than 1.5 mm, Oce_0: absence of ocelli, SCA_present: presence of scales, PAO_present: presence of post-antennal organ, TRI_present: presence of trichobothria, REP_parth: parthenogenetic reproduction.

1.3.5 Effect of cropping systems and practice intensity on Collembola individual traits

Collected Collembola had an average body length of $768 \pm 479 \mu\text{m}$ (186-2573 μm) in 2020 and $888 \pm 502 \mu\text{m}$ (204-5179 μm) in 2021. Measured length traits showed no significant difference between systems (Table S1.5). However, distribution of Collembola length traits differed regarding cropping systems and years (Figure 1.5). Body length distribution differed between Conv, CA and OA (Figure 1.5). A higher share of small Collembola was observed in CA and OA than in Conv in 2020 and inversely in Conv and OA than in CA in 2021. In addition, body length was similar in Conv-CA and Conv in 2020 but not in 2021. Furthermore, Conv-OA trait distribution looks more like OA in 2021 than in 2020, while CA-OA followed a similar pattern in both years, which does not resemble CA nor OA. The few collected Collembola in Conv-OA in 2020 were less than 1 mm long with only one individual above 2.5 mm. Head, antenna and leg length did not bring further information (Figure 1.5). Furca length was variable in all systems and allowed to observe

that all individuals had a furca in OA in 2020 and in Conv-OA and CA-OA in 2021 (i.e. absence of some poduromorpha species) (Figure 1.5).

Overall, measured traits were impacted by pesticide treatment intensity in 2020 and by tillage intensity in 2021, but not by fertilization intensity (Table 1.7, Figure S1.3). In 2020, body, leg and antenna length increased significantly when treatment intensity increased. In 2021, body length followed similar tendency regarding treatment intensity, and body, head and furca length decreased significantly when tillage intensity increased.

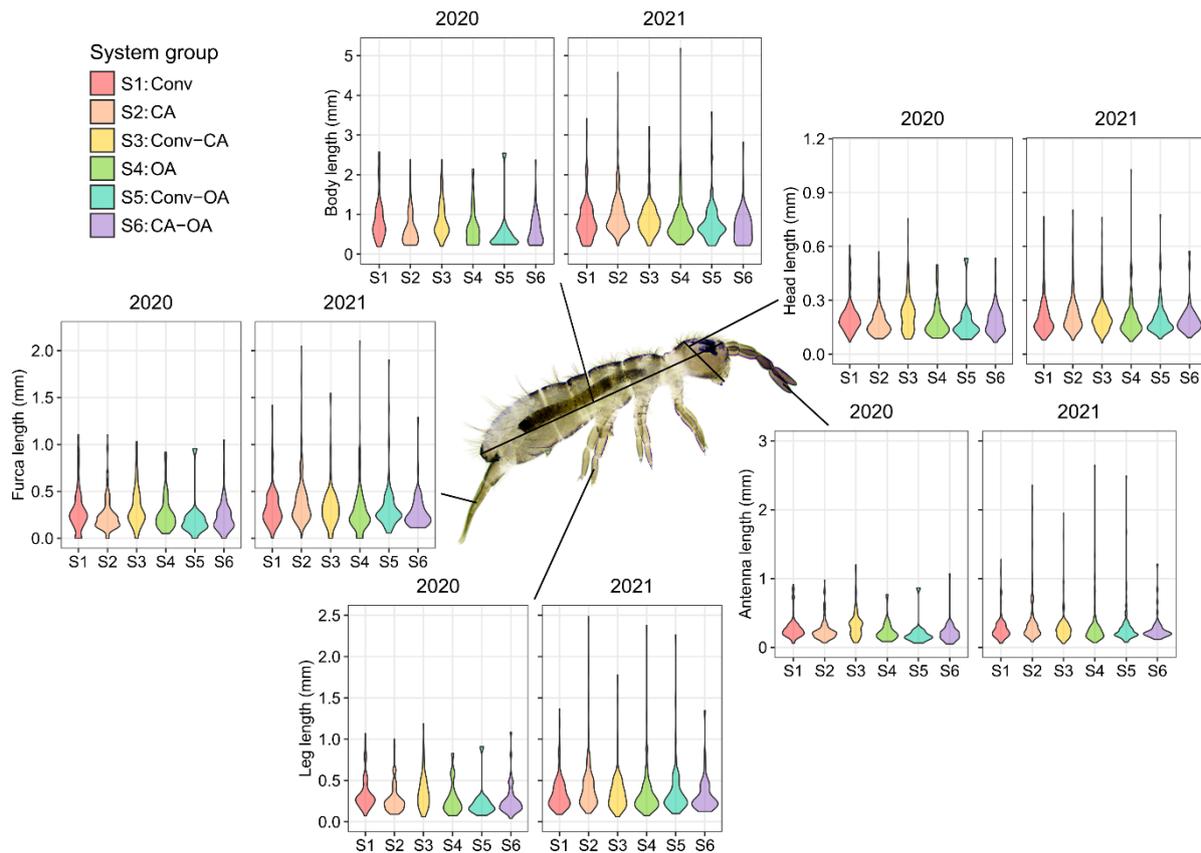


Figure 1. 5 Distribution of length traits measured on Collembola individuals in the different cropping systems in 2020 and 2021. Values are presented as violin plots. Width of violin represents the number of individuals presenting trait of a given length. Conv, CA and OA: conventional, conservation and organic agriculture; Conv-CA, Conv-OA and CA-OA: initial-recent system transitions.

Table 1. 7 Effect of practice intensity on measured length traits of Collembola in 2020 and 2021. z-value and P-value were obtained using GLMM with a negative binomial error distribution. Bold values indicate significant effect ($P < 0.05$ *, < 0.01 **, < 0.001 ***).

	2020						2021					
	Itill		Itreat		Ifertil		Itill		Itreat		Ifertil	
	z	P	z	P	z	P	z	P	z	P	z	P
M_BL	-0.84	0.400	2.22	0.026*	0.33	0.744	-2.90	0.004**	1.75	0.080	-0.22	0.826
M_HL	-0.69	0.492	1.78	0.075	-0.33	0.741	-2.68	0.007**	0.89	0.371	-0.33	0.740
M_LL	-1.13	0.259	2.52	0.012*	0.07	0.948	-1.77	0.077	0.58	0.560	-0.61	0.540
M_AL	-1.39	0.165	2.67	0.008**	0.03	0.979	-1.66	0.097	0.40	0.687	-0.59	0.554
M_FL	-0.89	0.372	1.55	0.120	-1.09	0.275	-2.59	0.009**	1.52	0.129	-0.58	0.562

Itill: tillage intensity index, Itreat: pesticide treatment intensity index, Ifertil: fertilization intensity index, M_BL: measured body length, M_HL: measured head length, M_LL: measured leg length, M_AL: measured antenna length, M_FL: measured furca length.

1.4 Discussion

1.4.1 Effect of cropping systems on soil mesofauna density: systems *versus* practice intensity

Our study showed that indexes representing practice intensity are useful to better explain the effect of cropping systems on soil mesofauna. Indeed, we observed differences in mesofauna density between cropping systems, but practice intensity indexes revealed effects that were hidden as plots were grouped into systems. They also helped to understand which practices are the most disturbing to soil mesofauna. In particular, some mesofauna groups (i.e. Actinidida Acari, euedaphic Collembola) were influenced only by one intensity index, reflecting a higher sensibility to one type of disturbance, which cannot be detected with the system approach.

The comparison between cropping systems showed that Collembola density tend to be higher in stable conservation systems than in organic systems, in line with results reported for other soil organisms (Henneron et al., 2015). However, even if we observed benefits of conservation systems, the absence of significant difference between conservation and conventional system contrasts with previous studies (Brennan et al., 2006; Coulibaly et al., 2022; Dulaurent et al., 2022). Surprisingly, our results do not point toward benefits of organic agriculture on soil mesofauna density, which contrast with most previous studies (Hole et al., 2005; Christel et al., 2021). Yet, the absence of difference between organic and conventional systems was already reported, in particular for Collembola (Alvarez et al., 2001; Filser et al., 2002). Negative or neutral effects of organic agriculture on soil mesofauna may be explained as soils under organic agriculture can show a higher disturbance level than conventional ones due to increased tillage intensity (Mazzoncini et al., 2010). In line with this, we observed that all organic plots have a high tillage intensity, while it was more contrasted for conventional systems. In addition, the variability of the effects of organic agriculture on soil mesofauna could be explained by a larger annual variability in organic systems than in other systems. Indeed, we observed a very low density of mesofauna in organic systems in 2020, but no significant difference in 2021.

Stable and recent alternative systems had similar mesofauna density, in particular regarding conservation systems. Indeed, recent and stable conservation systems had a similar mesofauna density, although slightly lower in recent systems, suggesting rapid changes in mesofauna communities after conversion. However, in recent organic systems, the mesofauna density remained low in both years and tended to be lower than in stable organic systems. We suggest that a transitory negative effect on mesofauna has occurred during the first year of the transition to organic systems, hence contradicting reported benefits on Collembola and Acari densities during the transition to organic agriculture (Werner and Dindal, 1990; Filser et al., 2002; Schrader et al., 2006). This negative effect could be related to a lower density of Collembola due to an

increase in competition or predation by other organisms following the transition to organic agriculture (Filser et al., 2002). Moreover, systems in transition from conservation to organic agriculture presented a very high Acari/Collembola ratio in both years (significant in 2020 only), which could indicate that they provide a better environment for soil mesofauna. However, it was mostly due to a very low Collembola density in both years and suggests that this type of transition may cause important changes on the soil mesofauna community. To our knowledge, no other studies were conducted on the effect of the transition from conservation to organic systems on soil biodiversity. Therefore, more research is required to assess changes occurring at a longer term and on other groups of soil organisms.

Further investigations on the effects of cropping systems using intensity indexes revealed that mesofauna density was higher under lower physical disturbances (i.e. tillage intensity) but not under lower chemical disturbances (i.e. pesticide treatment intensity) contrary to our original hypothesis. This is consistent with the higher density observed in conservation than in organic systems, the first being commonly associated to the absence of tillage and high pesticide treatment intensity, and the latter to the absence of treatments and high tillage intensity. In the literature, tillage was often reported to be the main factor impacting soil mesofauna (Ferraro and Ghera, 2007; Coudrain et al., 2016; Coulibaly et al., 2017), while pesticides were designated as a second order factor of influence on microarthropods (Cortet et al., 2002). In our study, we surprisingly found pesticide treatments to be positively related to soil mesofauna, including Collembola and Acari. Ferraro and Ghera (2007) observed similar results for Acari, while they reported negative effects on Collembola. Pesticide treatments can have both direct and indirect effects on soil organisms. Indirect effects are most likely at the origin of the positive effects we observed. First, the main pesticides used in field crops are herbicides, which cause an increase in dead weed biomass at the soil surface, providing more food for decomposers (Wardle et al., 1999). In addition, herbicides are known to be used as an energy source by some species of microorganisms (Neher, 1995). This may lead to an increase in microbial biomass with cascading effect on mesofauna consumers. However, the quantity of herbicides applied to the field (few g or L·ha⁻¹) represents a relatively little quantity of carbon. Furthermore, treatments may also decrease the pool of predators, hence increasing the number of mesofauna preys (Bengtsson et al., 2005). More generally, cropping systems rely on an equilibrium between tillage and pesticide treatment intensity to manage weeds and pests. Therefore, at least part of the positive effect of pesticide treatments may be explained by the negative effect of tillage which is higher under systems relying on low treatment intensity.

In contrast, mesofauna density was not responding to fertilization intensity. The absence of effect of fertilization on mesofauna was already reported regarding both organic matter and mineral nitrogen inputs (Kanal, 2004; Coudrain et al., 2016). In addition, fertilization was

demonstrated to have less effect on mesofauna than crop type (Bandyopadhyaya et al., 2002; Moos et al., 2020; Gergócs et al., 2022). In our study, only wheat was cultivated in 2020 and different wheat varieties are not expected to influence mesofauna density (Salmon et al., 2021). In 2021, we detected no effect of crop type but the number of cultivated species in the five-year rotation had a positive effect on the mesofauna density. This suggests that longer crop rotations may favor mesofauna, but with variable effects depending on years.

1.4.2 Taxonomic and functional responses of Collembola diversity to cropping systems

The functional approach of diversity brought additional insight on the effect of cropping systems on Collembola. Indeed, Collembola functional traits appear to complement information obtained with the taxonomic approach as reported in previous studies (Pey et al., 2014; Winck et al., 2017). Differences were observed by looking at traits separately rather than by observing functional community composition or functional indices. At the community level, Collembola species composition differed between organic and conservation systems, but mostly due to the absence or low occurrence of several species in organic systems, while functional composition was not significantly different between cropping systems as they poorly influenced traits. In addition, Collembola species and functional richness were both very low in recent organic systems in 2020, but this was mostly an effect of the very low abundance of mesofauna and of the underrepresentation of Collembola in the samples.

L. cyaneus and *P. alba* could be good representatives of the effect of tillage and pesticide treatments, whereas we found no species representing the different cropping systems. The density of several Collembola species tends to be negatively affected by tillage intensity and positively by pesticide treatment intensity, but *L. cyaneus* and *P. alba* were abundant in all systems and showed significant responses to practices in both years. However, species are subjected to spatio-temporal dynamics and studies usually report the abundance of different species, or do not reach this level of identification, which limits the determination of indicator species.

Several species traits responded to tillage or pesticide treatment intensities. Morphological traits that were observed under low tillage intensity (i.e. presence of ocelli and trichobothria) and high treatment intensity (i.e. scales) correspond to Collembola living at the soil surface and in open habitats (Salmon et al., 2014). This is in agreement with the effects we observed for epedaphic and hemiedaphic Collembola, and in particular for *L. cyaneus* and *P. alba* (i.e. respectively epedaphic and hemiedaphic species) as they both have ocelli, trichobothria and scales. All of this supports the fact that tillage represents a main disturbance for the Collembola community, in particular for surface species presenting specific traits. These species may be more sensitive to physical injuries caused by tillage and deprived of food as organic matter is buried

owing to morphological characteristics that are not adapted to forage in soil. In contrast, traits that benefited from high tillage intensity (i.e. absence of ocelli, and observed trends for presence of PAO and parthenogenetic reproduction) correspond to Collembola living deeper in soil (Salmon et al., 2014). This effect was not observed on euedaphic Collembola density, probably due to a low density, which is a general trend in croplands (Martins da Silva et al., 2016). In addition, no euedaphic species responded to tillage intensity in both years. Species adapted to soil may however indirectly benefit from tillage as, contrary to surface species, the mixing of organic in soil increases their access to food.

All measured Collembola length traits were highly correlated and had a similar response to practice intensity. Appendages (head, leg, antenna, furca) length did not provide further information compared to body length. Therefore, body length measurement seems to be sufficient to assess change in Collembola length traits within cropping systems. However, further investigation are required to confirm if length traits are responding differently to disturbances under other ecosystem type.

While species traits based on the literature provide an average trait value based on the global community of Collembola, individual traits measured on collected Collembola represent the specific trait pool of the local Collembola community. In this study, we found that individual traits measurements bring additional information to species traits. Indeed, we did not observed significant effect of cropping systems on body length using species traits, while traits measured on collected individuals demonstrated a significant effect of practice intensity on body length, and other length traits, in both years of the study. Collembola tended to be larger and to have larger appendages under low tillage and high pesticide treatment intensities. This is consistent with our initial hypothesis that larger Collembola are impacted by physical disturbances. However, benefits of pesticides are surprising as they are reported to increase mortality and decrease reproduction of Collembola (Joimel et al., 2022). We suggest that the size increase can be related to a lower proportion of juveniles due to a lower reproduction. In addition, predators were reported to decrease under high treatment intensity (Bengtsson et al., 2005; Galloway et al., 2021). Thus, a low predation pressure may accelerate recolonization of fields by large and highly mobile Collembola.

Furthermore, measured traits helped to observe intra-specific variations between years, which could reveal some climatic or management effects. Larger mean body length observed in 2021 is partly due to the presence of larger species such as *Orchesella villosa* (Geoffroy, 1762) and of larger *Lepidocyrtus lignorum* (Fabricius, 1793) individuals than in 2020. Trait measurements of a specific species would be highly relevant when a species dominates all plots within a study and is present at a high density. It was not the case here, and we did not observe any significant change in length traits at the species level.

1.4.3 Intensity indexes and other perspectives to unearth the effect of cropping systems on soil mesofauna

Our study showed that alternative cropping systems can have highly variable effects on soil mesofauna. The intensity indexes confirmed that at least part of this variability can be explained by the diversity of applied practices. Indeed, they revealed a gradient of practice intensity with a high variability of practices even within cropping systems (i.e. tillage in conventional and organic systems, pesticide treatments in conventional and conservation systems). It is noteworthy that primary indicators and intensity indexes recovered a similar system differentiation in our study than Büchi et al. (2019), thus showing the robustness of this approach even with both stable and transitioning systems. Moreover, whereas tillage and pesticide treatment intensities were particularly useful to better describe the intensity of disturbances in cropping systems, fertilization intensity mostly reflected the strategy of each farmer in terms of organic and mineral fertilization. In the studied regions there is little livestock farming, thus access to organic amendment is more difficult and farmers often rely on an opportunist strategy that implies a large variability of fertilization practices.

To go further, primary indicators and composite indexes used in this study do not take into account frequency and time at which practices were performed, or time gap between their implementation and sampling of soil organisms. For instance, fertilization effects on mesofauna may depend on the nature of the applied fertilizers (i.e. mineral or organic nature of fertilizers, type of synthetic or organic fertilizers) and the doses and frequency of application (Weil and Kroontje, 1979; Miyazawa et al., 2002; Song et al., 2016), which are not included in the composite indexes that we used. Similar statement can be done for tillage and pesticide treatment indexes. This information requires more data collection effort, which is complex to handle, but could be of importance for the development of future cropping systems. In addition, the interactions between the three composite indexes did not reveal any effect on mesofauna density due to the necessarily low number of samples. Yet, it cannot be excluded that the interactions between different practices can influence mesofauna density. Indeed, some beneficial practices, such as low tillage, could be counterbalanced by other practices. For instance, combined tillage and organic matter input are reported to benefit soil organisms (Jabbour et al., 2016), in particular Acari (Miyazawa et al., 2002). Therefore, we encourage future studies to assess conjointly the effect of practices to further our understanding of the effects of the cropping systems on soil biodiversity.

In addition, our results were also impacted by a large inter-annual variability in mesofauna density and diversity, as it was often reported for soil organisms, especially for microarthropods (Tabaglio et al., 2009; Gergócs et al., 2022). These variations may be partly due to climatic conditions (Taylor et al., 2004; Salmon et al., 2021). However, the effects of climatic conditions

could be more or less important depending on cropping systems (Meyer et al., 2021). In addition, seasonal variability is reported to be even larger than variability between years (Berg and Bengtsson, 2007) and could be especially important in transitioning systems regarding Collembola (Werner and Dindal, 1990). Long-term studies are required to assess more precisely the effects of cropping systems on soil mesofauna, with sampling points both in autumn and in spring when soil mesofauna is reported to be more active.

1.5 Conclusion

Conventional, organic and conservation agriculture differ broadly in terms of tillage, pesticide treatments and fertilization, but the great variability observed *in situ* within each of these practices makes difficult to compare cropping systems and to study their effects on soil biodiversity. Therefore, taking into account the intensity of various practices *via* composite indexes seems relevant to better understand the effects of cropping systems on soil organisms, especially mesofauna, with the long-term objective to develop alternative and agroecological systems relying on strong soil biodiversity.

Furthermore, taxonomic and functional trait approaches of diversity bring complementary insight on the way Collembola communities are influenced by practice intensity. We therefore encourage their combined use in order to assess the diversity of Collembola in cropping systems. Moreover, Collembola species traits available in existing databases and individual traits measured in the specific context of the study are both useful to observe how communities respond to soil disturbances. Measurement of length traits is especially relevant to assess more accurately the effect of practices on the local Collembola community. However, because all measured length traits followed the same trends, and as trait measurement is undoubtedly time consuming, we suggest future studies in agricultural areas to concentrate their effort first and foremost on body length measurements.

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———— Chapter 2 ————

Practice intensity influences specific groups of soil macrofauna in cropping systems

Juliette Chassain¹, Sophie Joimel¹, Antoine Gardarin², Laure Vieublé Gonod¹

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¹ Université Paris-Saclay, INRAE, AgroParisTech, UMR ECOSYS, 91120, Palaiseau, France

² Université Paris-Saclay, INRAE, AgroParisTech, UMR Agronomie, 91120, Palaiseau, France

Abstract

Larger soil organisms have been reported to be the most sensitive to disturbances caused by practices in cropping systems. However, soil macrofauna comprises groups with a wide diversity of morphological and ecological features, which may respond differently to applied practices. Here, we aim to assess the effects of different cropping systems and practice intensity on soil macroarthropods and earthworms. Macrofauna organisms were extracted from soil blocks in autumn 2020 and 2021 over 21 plots belonging to conventional, conservation or organic systems, either stable (≥ 7 years) or in transition (≤ 3 years). Composite indexes of practice intensity were calculated to assess the intensity of tillage, pesticide treatments and fertilization practices. The density of macrofauna groups, earthworm species richness and earthworm functional traits were investigated on each plot.

The density and diversity of macroarthropods and earthworms demonstrated few differences regarding different cropping systems and practice intensity, with effects varying across groups and years. Specific macroarthropod groups responded differently to tillage, treatment and fertilization intensity. In addition, high tillage intensity had a negative effect on the density and biomass of epi-anecic earthworm juveniles and earthworm species with a small body size. High fertilization intensity had a negative effect on the density and biomass of epi-anecic earthworms and a positive effect on endogeic adults, and could be related to several earthworm functional traits (body length, mass/length ratio, epithelium type, carbon preferences). Taxonomic and functional traits approaches of earthworm community led to similar conclusions. Overall, our results support the need to account for the actual intensity of practices and for different taxonomic, trophic and ecological groups in order to better assess the effect of cropping systems on soil macrofauna.

2.1 Introduction

Intensive management of agroecosystems is a major cause of soil biodiversity loss (Gardi et al., 2013). Some groups of soil organisms could respond faster to disturbances caused by agricultural practices in relation to their size or their trophic position (Coudrain et al., 2016; Coller et al., 2022). In particular, larger organisms were shown to be the most impacted by agricultural intensification and to be more sensitive to disturbances caused by practices (Postma-Blaauw et al., 2010, 2012).

Soil macrofauna comprises the largest soil invertebrates (> 2 mm) and many taxa (Lavelle and Spain, 2001). Macroarthropods and earthworms are the two main groups considered in studies assessing soil macrofauna. However, while macroarthropods are mostly living at the soil surface, except during their larval stage, earthworms are the main belowground actors of macrofauna. Macroarthropods are involved in several functions at the soil surface such as litter decomposition (Frouz, 2018; Chassain et al., 2021) and pest regulation through predation (Kromp, 1999). Among them, the extensively studied Carabidae (i.e. ground beetles) are considered as useful bioindicators to compare different cropping systems (Burgio et al., 2015). On the other hand, earthworms have long been recognized as playing a major role in soil functioning. Indeed, Darwin stated that the ability of earthworms to mix soil and surface residues makes them a natural counterpart of tillage (Feller et al., 2003). Earthworms are important drivers of soil properties and functioning through their activities of burrowing, tunneling, feeding and casting, that influences organic matter decomposition (Barrios, 2007) and stabilization (Bertrand et al., 2015), microbial activity (Kladivko, 2001) and soil structure (Joschko et al., 1989; Young et al., 1998). Nowadays, they are often considered as bioindicators of soil quality (Pérès et al., 2011), soil biodiversity (Bispo et al., 2009) and soil disturbances caused by different cropping systems (Paoletti, 1999; Masin et al., 2020).

Conventional cropping systems are reported to have negative effects on soil macrofauna density, biomass, diversity and activity (Eggleton et al., 2005; Yin et al., 2022), particularly on earthworms (Young and Ritz, 2000; Tsiafouli et al., 2015; Briones and Schmidt, 2017). Alternative systems, which were developed to offset the drawbacks of intensive agriculture, may benefit the abundance of several taxonomic and functional groups of soil macrofauna (Henneron et al., 2015; Pelosi et al., 2015; Tahat et al., 2020). In particular, organic systems demonstrated positive effects on total macroarthropod density compared to conventional systems (Maeder et al., 2002; Hole et al., 2005), but opposite results were also reported with variable responses according to taxonomic groups (Henneron et al., 2015; Patterson et al., 2019; Christel et al., 2021). Similarly, the density, biomass and diversity of earthworms were alternatively reported to be higher (Bettiol et al., 2002; Maeder et al., 2002; Hole et al., 2005; Pelosi et al., 2015) or lower (Bengtsson et al., 2005; Hole et

al., 2005) in organic than in conventional systems. Conservation agriculture also demonstrated benefits on soil macrofauna, in particular on earthworm density and biomass (Mele and Carter, 1999; Hernández et al., 2017; Dulaurent et al., 2022). However, few studies have been conducted on the effects of conservation systems on soil biodiversity (Christel et al., 2021), which prevents to assess the effective variability in the responses of soil organisms to conservation agriculture.

Furthermore, few studies assessed the effect of the transition from conventional to alternative systems, or from one alternative to the other. The conversion to organic agriculture was found to benefit ground-dwelling macroarthropod abundance during the first years of the transition (Lundgren et al., 2006), but depending on the practices applied at the beginning of the transition (Schipanski et al., 2014; Jabbour et al., 2016; Gareau et al., 2019) and on taxa (Tsutsui et al., 2018). Similarly, earthworm density reportedly increases in the first year of the transition to organic (Irmiler, 2010) and to no-tillage systems (Stubbs et al., 2004). However, this increase might be transitory (Pelosi et al., 2015, 2016). Even fewer studies assessed the consequences of the transition to hybrid systems such as combined organic and no-tillage systems. These latter systems were reported to have variables effects on earthworms and macroarthropods (Peigné et al., 2018; Rivers et al., 2020; Boeraeve et al., 2022).

Macrofauna organisms are influenced by a wide range of cropping practices having positive or negative effects on the density or diversity of specific groups. Macroarthropods were reported to respond unevenly to tillage (Stubbs et al., 2004; Patterson et al., 2019), crop rotation (Patterson et al., 2019), organic matter inputs (de Souza et al., 2016; Růžičková et al., 2020), and pesticide treatments (Wardle et al., 1999; Burgio et al., 2015; Pearsons and Tooker, 2021). Numerous studies demonstrated a negative effect of tillage on earthworm abundance (Wardle, 1995; Roger-Estrade et al., 2010), but the absence of significant effect was also reported (Capowiez et al., 2009a). Regarding earthworms, the influence of tillage can be modulated by soil properties and varies for different ecological categories (Chan, 2001). In addition, pesticides reportedly have a negative effect on earthworm density (Pelosi et al., 2014a; Datta et al., 2016), while organic matter inputs benefit earthworm density (Birkhofer et al., 2008; Ponge et al., 2013; Bertrand et al., 2015) and biomass (Capowiez et al., 2009b; D'Hose et al., 2018).

Overall, the effects of cropping systems and practices on soil macrofauna appear to be highly variable, with previous studies showing inconsistent results. Hence, the development of alternative systems relying on strong soil macrofauna density and diversity requires a finer level of description than the current focus on system categories or single practices (Roger-Estrade et al., 2010). To that aim, some authors already emphasized the interest of using indicators on the intensity and frequency of soil disturbances to compare the effect of different management intensities on soil macrofauna (Gareau et al., 2019; Masin et al., 2020). Indicators proved to be useful to monitor soil health and earthworm populations (Masin et al., 2020). In this study, we

used indicators of practice intensity adapted from Büchi et al. (2019) in order to better assess the effects of different cropping systems, stable or in transition, on soil macrofauna.

Furthermore, functional traits are increasingly used to understand the responses of the soil community to disturbances such as the ones caused by agricultural practices (Hedde et al., 2012; Pey et al., 2014; deCastro-Arrazola et al., 2022). Indeed, traits or functional indexes may provide a better overview of the effects of soil disturbances on soil fauna than species diversity (Pelosi et al., 2014). The relations between agricultural practices and macrofauna traits were mostly studied for ground beetles (Cole et al., 2002; Liu et al., 2012; Boinot et al., 2019) and in a lesser extent for earthworms (Pelosi et al., 2014; Pelosi et al., 2016; Frazão et al., 2019). In this study, we assessed the effects of cropping systems and practice intensity on earthworm diversity using both species and functional approaches.

The objectives of our study were 1) to assess the effects of different cropping systems, stable or in transition, on soil macrofauna, 2) to test if the use of indicators of practice intensity (tillage, pesticide treatments, fertilization) allows for a better characterization of the effects of cropping systems, and 3) to investigate the relevance of the functional approach to determine the effects of disturbances on earthworm diversity compared to the sole taxonomic identification. We hypothesized that systems with lower physical (i.e. tillage) and chemical (i.e. pesticide treatments) disturbances present higher density and diversity of macrofauna as well as a modified community composition compared to intensive systems. In addition, we expected negative impacts of high tillage and pesticide treatment intensities on total soil macrofauna, and a variable effect of fertilization depending on groups of earthworms and macroarthropods (i.e. detritivores). Lastly, earthworm species and functional traits were expected to vary according to practice intensity, with in particular larger earthworms under lower tillage intensity.

2.2 Materials and methods

2.2.1 Study sites

The study was conducted over 21 plots owned by farmers and cropped with winter wheat in 2020-2021, and on the same plots but with various crops or cover crop in 2021-2022 (Table 2.1). All plots are located in the Paris area (Yvelines, Eure-et-Loir and Essonne departments), France. Climate is temperate with mean annual precipitations of 600 to 700 mm and a mean annual temperature of 11°C (cf. General introduction for more details). Soils were silty or clayed, with a pH ranging from 5.6 to 8.1 and a bulk density varying between 1.1 and 1.5 g.cm⁻³ at 0-10 cm depth (Table 2.1).

Table 2. 1 Location, crop, cropping system and soil parameters (0-10 cm depth) of the study plots. Age corresponds to the number of years since conversion to the current system in 2020.

Plot	Region	Crop 2020	Crop 2021	System	Age	Clay	Silt	Sand	SOM	pH	BD
						g.kg ⁻¹	g.kg ⁻¹	g.kg ⁻¹	g.kg ⁻¹		
A1	Eure-et-Loir	wheat	wheat	Conv-CA	3	151.3	474.3	55.7	19.5	6.8	1.3
A2	Essonne	wheat	cover	CA	17	210.3	381.3	118.7	33.5	6.3	1.3
A3	Essonne	wheat	wheat	OA	20	157.7	409.0	196.3	17.5	6.5	1.4
A4	Yvelines	wheat	bare	OA	19	426.3	236.7	93.3	34.4	7.9	1.1
A5	Eure-et-Loir	wheat	barley	Conv	20	206.0	448.0	52.0	24.5	6.8	1.3
A6	Essonne	wheat	bare	CA-OA	2	277.3	377.7	28.7	21.0	6.6	1.3
A7	Yvelines	wheat	rye	Conv-OA	2	172.3	481.3	63.0	22.3	6.6	1.2
A8	Essonne	wheat	mustard	CA	7	192.7	363.7	182.7	24.0	5.6	1.5
A9	Yvelines	wheat	rapeseed	Conv	20	208.0	402.3	187.3	18.5	6.8	1.3
A10	Eure-et-Loir	wheat	alfalfa	Conv	20	219.7	441.7	46.7	20.9	7.2	1.3
A11	Yvelines	wheat	cover	OA	20	252.7	350.0	120.3	27.0	7.3	1.2
A12	Yvelines	wheat	cover	Conv	20	221.3	352.0	164.3	23.3	7.4	1.3
A13	Yvelines	wheat	rye, lentil	CA-OA	2	248.3	411.7	75.3	33.4	6.9	1.2
A14	Yvelines	wheat	cover	Conv-CA	3	175.0	385.0	119.7	32.0	6.8	1.2
A15	Yvelines	wheat	wheat	Conv-CA	3	193.3	395.3	91.7	28.1	7.0	1.4
A16	Essonne	wheat	mustard	Conv	20	284.3	338.0	66.7	24.2	8.1	1.4
A17	Yvelines	wheat	rapeseed	CA	10	228.0	349.0	203.3	36.4	8.1	1.2
A18	Yvelines	wheat	bare	Conv-OA	3	195.7	454.7	68.3	23.9	6.8	1.2
A19	Yvelines	wheat	clover	Conv-OA	2	279.7	264.0	262.0	23.8	7.7	1.3
A20	Eure-et-Loir	wheat	cover	CA-OA	2	204.0	407.0	49.3	16.9	7.5	1.4
A21	Eure-et-Loir	wheat	barley	Conv	20	340.0	226.3	238.0	32.9	8.0	1.2

Conv: conventional agriculture, CA: conservation agriculture, OA: organic agriculture, Conv-CA: transition from conventional to conservation (≤ 3 years), Conv-OA: transition from conventional to organic (≤ 3 years), CA-OA: transition from conservation to organic (≤ 3 years), SOM: soil organic matter, BD: bulk density.

2.2.2 Agricultural practices and intensity indexes

Each of the 21 plots were assigned to one of the six cropping systems defined as follows: i) three stable (≥ 7 years) systems under conventional (Conv, $n = 6$ plots), organic (OA, $n = 3$) or conservation agriculture (CA, $n = 3$) and ii) three systems in transition (≤ 3 years) from conventional to organic (Conv-OA, $n = 3$), conventional to conservation (Conv-CA, $n = 3$) or conservation to organic agriculture (CA-OA, $n = 3$) (Table 2.1).

All information regarding practices applied to each plot (e.g. tillage, fertilization, pesticide treatments, crop rotation, dates of seedling and harvest) were collected by conducting a survey amongst the farmers. Primary indicators and composite indexes of practice intensity were selected from Büchi et al. (2019) to describe crop rotation, tillage, pesticide treatments and fertilization practices in each plot (Table 2.2). The composite indexes of tillage intensity (Itill) and fertilization intensity (Ifertil) were computed by an additive combination of the normalized values of primary indicators (i.e. nbDTill, nbSTill, soilP for Itill and nbOrg, qminN, resExp for Ifertil). The composite index for pesticide treatment intensity (Itreat) was the normalized value of the total number of applied treatments (cf. General introduction). Obtained values for primary indicators and composite indexes in 2020 and in 2021 are reported in Table 2.2.

Table 2.2 Primary indicators and composite indexes of practice intensity used to characterize cropping systems. Values are presented for 2020 and 2021. Composite indexes are given in bold. Primary indicators were computed for practices occurring in one year or in five years for nbCrop, soilP, nbOrg, resExp.

Name	Description	Mean value in cropping system (2020 ; 2021)					
		Conv	CA	Conv-CA	OA	Conv-OA	CA-OA
nbCrop	Crops in the rotation	3.2 ; 3.5	3.3 ; 3.7	3.7 ; 3.7	3.7 ; 3.7	3 ; 3.7	3.3 ; 3.7
nbDTill	Deep tillage interventions (> 15 cm)	0.7 ; 0.7	0 ; 0	0 ; 0	1.7 ; 1.3	1.7 ; 1.3	1 ; 0.3
nbSTill	Surface tillage interventions (< 15 cm)	5.3 ; 4	0.7 ; 0	0.3 ; 0.3	6.3 ; 7.7	3.7 ; 7	7 ; 8.3
soilP	Type of tillage (plough = 0.5, no-till = 5)	2 ; 2	4.9 ; 4.9	3.8 ; 4.1	1.3 ; 1.3	1.5 ; 1.5	2.3 ; 2.3
nbHerb	Herbicide treatment applications	5.2 ; 7.8	6.3 ; 6	5.3 ; 6.3	0 ; 0	0 ; 0	0 ; 0
nbFung	Fungicide treatment applications	0.8 ; 1.8	0.3 ; 1.7	1 ; 3.3	0 ; 0	0 ; 0	0 ; 0
nbInsect	Insecticide treatment applications	1.3 ; 0.8	0 ; 0.7	2.3 ; 2	0 ; 0	0 ; 0	0 ; 0
nbOrg	Organic matter inputs	0.8 ; 0.8	0.7 ; 0.7	1 ; 1	5 ; 5	3.3 ; 3.3	2.3 ; 3
qminN	Mineral N fertilizer inputs (kg N.ha ⁻¹)	129 ; 198	109 ; 130	69 ; 187	0 ; 0	0 ; 0	0 ; 0
resExp	Residues exportations	0.3 ; 0.3	0 ; 0	2.3 ; 2.3	1 ; 1	2.3 ; 2.3	0 ; 0.3
Itill	Tillage intensity	0.5 ; 0.4	0 ; 0	0.1 ; 0.1	0.7 ; 0.7	0.6 ; 0.6	0.6 ; 0.4
Itreat	Pesticide treatment intensity	0.7 ; 0.8	0.6 ; 0.6	0.8 ; 0.8	0 ; 0	0 ; 0	0 ; 0
Ifertil	Fertilization intensity	0.3 ; 0.3	0.2 ; 0.2	0.4 ; 0.5	0.4 ; 0.4	0.4 ; 0.4	0.1 ; 0.2

Conv, CA and OA: conventional, conservation and organic agriculture, Conv-CA, Conv-OA, CA-OA: initial-recent system transitions.

2.2.3 Soil sampling and physico-chemical analyses

Soil samples were collected in autumn 2020 from October 19th to December 2nd, and in autumn 2021 from October 25th to November 22nd. They took place minimum one week after sowing to allow for a partial recovery of soil organisms after mechanical operations. Three replicates spaced by 25 m were defined in each plot. They were set 25 m apart from the field margins and outside of tractor traffic tracks to avoid bias.

Composite soil samples were collected on each replicate at 0-10 and 10-20 cm depth by mixing eight soil cores obtained with an auger. A fraction of soil sampled in 2020 was sieved at 4 mm, air dried and analyzed by the INRAE Laboratory of Arras to characterize the main soil properties (cf. General introduction).

2.2.4 Macrofauna sampling

Three soil blocks of 25 x 25 x 25 cm were extracted on each plot and hand-sorted on the field to collect earthworms and other macrofauna organisms. Earthworms were preserved in 4% formol and other organisms in 70% ethanol.

All macrofauna organisms were identified at the order level under binoculars. Coleoptera and Hemiptera were further identified at the family level. Ants were collected but not counted and not included in the analysis owing to their specific status of social insects. Earthworms were sorted depending on their development stage (adults, sub-adults, juveniles) and their ecological category (endogeic, epi-anecic, epigeic, intermediate). Ecological categories were determined following the initial categories defined by Bouché (1972, 1977) and revisited by Bottinelli et al. (2020). Adults

and sub-adults were identified at the species level. Then, earthworms were weighed by species or by groups to obtain the average biomass ($\text{g}\cdot\text{m}^{-2}$). The taxonomic diversity of earthworms was estimated with species richness and by calculating Shannon and Pielou's evenness indexes.

The assessment of ecological categories of earthworms was refined by using for each species the percentages by which they belong to the three main ecological categories (anecic, endogeic, epigeic) as computed in Bottinelli et al. (2020) (Table S2.1). We considered these three categories as three attributes of the same variable and calculated the community-weighted means (CWM). This conducts to obtain a fuzzy estimate of the share of the different ecological categories in the community of adult earthworms (as only adults were identified at the species level).

2.2.5 Earthworm functional traits

Earthworm functional traits were selected as parameters that could be related to the effect of practices and associated changes in soil properties. We thus selected four morphological (body length, body mass/length ratio, cocoon diameter, epithelium type), one ecological (carbon preferences) and one behavioral traits (vertical distribution in soil) (Pelosi et al., 2014; Briones and Álvarez-Otero, 2018; Frazão et al., 2019). The body length and cocoon diameter may indicate the impact of physical disturbances, as the larger the earthworms or cocoon are, the more they risk to be impacted by soil physical disturbances such as tillage (Pelosi et al., 2014; Frazão et al., 2019). The mass/length ratio can be an indicator of the growth and maintenance of earthworms in the field. Epithelium type represents the elasticity or strength of earthworm's skin, thus its resistance to different types of pressures, and may respond to tillage intensity (Pelosi et al., 2014; Frazão et al., 2019). Species with a preference for environments that are rich in organic carbon are expected to be present in fields with a high quantity of organic matter and higher fertilization intensity. Finally, the vertical distribution in soil may help to determine the depth at which different practices are impacting soil organisms. All traits for earthworm's species were collected from the BETSI database (CESAB/FRB) (Pey et al., 2014). Trait values for each species are reported in Table S2.2.

For each trait, the community-weighted means (CWM) were calculated as the weighted mean of trait classes in communities (Lavorel et al., 2008). Functional richness (Fric) and evenness (Feve) were computed as indexes of the functional diversity of earthworms (Villéger et al., 2008).

2.2.6 Statistical analyses

Prior to analyses, the three replicates were pooled to obtain one value per plot. The macrofauna density was obtained by dividing abundance data by the area of extraction (0.0625 m²). The normality and homogeneity of variances were tested with a Shapiro-Wilk test ($\alpha > 0.05$) and a Bartlett test ($\alpha > 0.05$). Relations between cropping systems or practice intensity and soil macrofauna parameters (i.e. macrofauna density and diversity, earthworm biomass, functional traits and percentages of belonging to ecological categories) were assessed using generalized linear models (GLM) and the quasi-Poisson family to account for the non-normal distribution and overdispersion of data, followed by Tukey HSD post-hoc tests when comparing different systems.

A principal component analysis (PCA) was performed on the main macrofauna groups in order to assess the macrofauna community composition in different cropping systems. Differences in the composition of the earthworm species communities between cropping systems were assessed using a principal coordinates analysis (PCoA) with Bray-Curtis distance, followed by a permutational analysis of similarities (ANOSIM) to test for significant differences in composition. Species occurring only once were removed for the analysis.

All the analyses were performed using R software version 3.6.3 (R Development Core Team 2020) and the *stats* (R Development Core Team 2020), *vegan* (Oksanen et al., 2022), *ade4* (Dray and Dufour, 2007), *FD* (Laliberté et al., 2022) and *multcomp* (Hothorn et al., 2022) packages.

2.3 Results

2.3.1 Cropping system effects on macrofauna (excluding earthworms)

Seven classes including 13 orders of macrofauna (without earthworms) were collected in 2020 and 2021 (Table 2.3a). The mean density of total macrofauna was 89 ind.m⁻² and 131 ind.m⁻² in 2020 and 2021 respectively, or 88 ind.m⁻² and 123 ind.m⁻² when accounting for macroarthropods only. The main macroarthropod groups were Coleoptera (larvae, Carabidae, Staphylinidae, Curculionidae), Diplopoda (Julida, Polydesmida), Araneae and Chilopoda (Geophilomorpha, Lithobiomorpha) (Table 2.3a). Gastropoda (mostly Limacidae) were collected in many plots in 2021 (Table 2.3a).

The total macrofauna density and number of orders did not differ significantly between cropping systems (Table 2.3a and b). In 2020, we observed a significantly higher density of Chilopoda in CA than in CA-OA and of Coleoptera larvae in CA than in Conv (Table 2.3a). In 2021, the density of Carabidae was significantly higher in Conv-CA than in Conv and OA (Table 2.3a).

Table 2. 3 Macrofauna (excluding earthworms) (a) density (ind.m⁻²) and (b) diversity in different cropping systems in 2020 and 2021. Values are means \pm standard deviations. Different lower-case letters and values in bold indicate significant differences between cropping systems according to the results of GLM with a quasi-Poisson error distribution ($P < 0.05$).

Taxa	2020						2021					
	Conv	CA	Conv-CA	OA	Conv-OA	CA-OA	Conv	CA	Conv-CA	OA	Conv-OA	CA-OA
(a) Density												
Total	75 \pm 37	153 \pm 64	94 \pm 61	133 \pm 162	43 \pm 23	53 \pm 9	114 \pm 95	165 \pm 67	149 \pm 67	155 \pm 91	89 \pm 99	132 \pm 62
Araneae	9 \pm 12	23 \pm 13	11 \pm 9	7 \pm 3	4 \pm 6	2 \pm 3	36 \pm 22	36 \pm 3	16 \pm 11	23 \pm 11	18 \pm 22	16 \pm 14
Chilopoda	6 \pm 5	21 \pm 14	9 \pm 6	7 \pm 12	2 \pm 3	0 \pm 0	15 \pm 29	7 \pm 8	20 \pm 29	5 \pm 9	7 \pm 12	5 \pm 5
	ab	a	ab	ab	ab	b						
<i>Geophilomorpha</i>	4 \pm 6	16 \pm 16	7 \pm 8	5 \pm 9	2 \pm 3	0 \pm 0	3 \pm 4	5 \pm 9	7 \pm 12	5 \pm 9	7 \pm 12	5 \pm 5
<i>Lithobiomorpha</i>	2 \pm 3	5 \pm 5	2 \pm 3	2 \pm 3	0 \pm 0	0 \pm 0	12 \pm 25	2 \pm 3	12 \pm 17	0 \pm 0	0 \pm 0	0 \pm 0
Coleoptera	25 \pm 20	80 \pm 30	64 \pm 46	48 \pm 38	27 \pm 11	34 \pm 20	43 \pm 45	82 \pm 40	85 \pm 44	50 \pm 13	59 \pm 56	69 \pm 46
<i>Carabidae</i>	9 \pm 12	14 \pm 16	11 \pm 11	18 \pm 16	7 \pm 8	7 \pm 8	11 \pm 6	14 \pm 3	34 \pm 13	12 \pm 6	18 \pm 8	21 \pm 11
							b	ab	a	b	ab	ab
<i>Curculionidae</i>	3 \pm 3	5 \pm 5	4 \pm 3	2 \pm 3	0 \pm 0	0 \pm 0	2 \pm 4	0 \pm 0	0 \pm 0	0 \pm 0	2 \pm 3	4 \pm 3
<i>Staphylinidae</i>	6 \pm 9	4 \pm 3	5 \pm 5	7 \pm 8	11 \pm 0	2 \pm 3	19 \pm 38	18 \pm 11	20 \pm 8	14 \pm 13	5 \pm 5	16 \pm 19
<i>Other</i>	2 \pm 3	5 \pm 5	0 \pm 0	2 \pm 3	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0
<i>Larvae</i>	5 \pm 9	52 \pm 16	44 \pm 39	20 \pm 20	9 \pm 3	25 \pm 17	12 \pm 11	50 \pm 36	32 \pm 30	23 \pm 22	34 \pm 54	28 \pm 40
	b	a	ab	ab	ab	ab						
Dermoptera	1 \pm 2	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	4 \pm 6	0 \pm 0	2 \pm 3	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0
Diplopoda	30 \pm 27	4 \pm 6	4 \pm 6	69 \pm 116	4 \pm 6	14 \pm 20	14 \pm 23	5 \pm 9	9 \pm 15	68 \pm 99	2 \pm 3	37 \pm 30
<i>Julida</i>	11 \pm 16	4 \pm 6	2 \pm 3	30 \pm 48	0 \pm 0	12 \pm 17	5 \pm 13	5 \pm 9	4 \pm 6	36 \pm 44	2 \pm 3	27 \pm 27
<i>Polydesmida</i>	20 \pm 28	0 \pm 0	2 \pm 3	39 \pm 68	4 \pm 6	2 \pm 3	9 \pm 22	0 \pm 0	5 \pm 9	32 \pm 55	0 \pm 0	11 \pm 5
Diplura	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	5 \pm 9	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0
Diptera (larv.)	4 \pm 4	12 \pm 22	2 \pm 3	0 \pm 0	0 \pm 0	0 \pm 0	1 \pm 2	2 \pm 3	5 \pm 5	0 \pm 0	0 \pm 0	2 \pm 3
Gastropoda	0 \pm 0	7 \pm 12	2 \pm 3	2 \pm 3	0 \pm 0	0 \pm 0	4 \pm 6	20 \pm 13	12 \pm 11	9 \pm 8	4 \pm 6	2 \pm 3
Hemiptera	0 \pm 0	0 \pm 0	2 \pm 3	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	4 \pm 3	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0
Isopoda	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	2 \pm 3	0 \pm 0	0 \pm 0	7 \pm 12	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0
Lepidoptera (larv.)	0 \pm 0	5 \pm 9	2 \pm 3	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	2 \pm 3	2 \pm 3	0 \pm 0	0 \pm 0	0 \pm 0
(b) Diversity												
Number of orders	4 \pm 1	5 \pm 2	5 \pm 1	4 \pm 2	3 \pm 2	3 \pm 2	4 \pm 2	6 \pm 1	5 \pm 2	4 \pm 1	3 \pm 2	5 \pm 2

Conv, CA and OA: conventional, conservation and organic agriculture, Conv-CA, Conv-OA, CA-OA: initial-recent system transitions, larv.: larvae.

Table 2. 4 Effect of practice intensity on macrofauna (excluding earthworms) (a) density (ind.m⁻²) and (b) diversity in 2020 and 2021. *t*-value and *P*-value were obtained using GLM with a quasi-Poisson error distribution. Bold values indicate significant effects ($P < 0.05$ *, < 0.01 **, < 0.001 ***). Primary indicators having a significant ($P < 0.05$) positive (+) or negative (-) effect on density are reported.

	2020						2021						Indicators 2020	Indicators 2021
	Itill		Itreat		Ifertil		Itill		Itreat		Ifertil			
	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>		
(a) Density														
Total	-0.66	0.516	0.94	0.359	0.73	0.477	-1.77	0.093	0.72	0.478	-0.69	0.500	n.s.	(-) nbDTill
Araneae	-1.94	0.067	2.66	0.015*	-1.38	0.184	-1.30	0.209	1.50	0.150	-1.58	0.132	(+) nbHerb; (-) nbSTill	(-) resExp
Chilopoda	-1.47	0.158	2.09	0.050	0.11	0.912	-3.04	0.007**	1.66	0.112	-2.91	0.009**	(+) nbHerb, nbCrop; (-) nbDTill, resExp	
													(-) nbSTill	
<i>Geophilomorpha</i>	-1.51	0.149	1.89	0.074	0.46	0.649	-0.64	0.532	0.25	0.809	-3.41	0.003**	(+) nbHerb; (-) nbSTill	(-) nbInsect, resExp
<i>Lithobiomorpha</i>	-0.62	0.545	1.30	0.208	-0.41	0.689	-2.09	0.050	2.77	0.012*	-1.73	0.101	(+) nbCrop; (-) nbSTill	(+) nbHerb, nbFung, qminN; (-) nbOrg
Diplopoda	1.93	0.069	-0.76	0.456	0.66	0.517	1.32	0.203	-1.09	0.292	0.68	0.507	n.s.	(+) nbOrg; (-) nbInsect, qminN
<i>Julida</i>	1.63	0.120	-2.64	0.016*	-0.48	0.635	1.08	0.293	-1.29	0.211	0.37	0.718	(+) nbHerb	(+) nbOrg; (-) nbInsect
<i>Polydesmida</i>	2.27	0.035*	-0.22	0.828	2.34	0.030*	1.48	0.154	-0.65	0.523	0.91	0.374	(+) nbCrop; (-) nbSTill	(+) soilP, resExp; (-) qminN
Coleoptera	-1.78	0.091	1.18	0.254	0.78	0.444	-2.00	0.060	0.53	0.605	-0.84	0.410	n.s.	n.s.
<i>Carabidae</i>	-0.50	0.624	0.57	0.577	1.77	0.092	-2.01	0.059	0.03	0.975	0.83	0.419	n.s.	n.s.
<i>Curculionidae</i>	-1.40	0.179	1.59	0.129	-3.24	0.004**	2.23	0.038*	-1.80	0.088	0.40	0.696	(+) nbCrop; (-) resExp	(+) soilP, nbFung; (-) nbCrop
<i>Staphylinidae</i>	0.87	0.393	0.32	0.754	2.96	0.008**	-1.83	0.082	1.20	0.245	-1.08	0.295	(-) nbSTill	n.s.
<i>Larvae</i>	-1.85	0.080	0.87	0.395	-0.26	0.798	-0.74	0.465	-0.08	0.938	-0.85	0.406	n.s.	(-) nbInsect
(b) Diversity														
Nb orders	-1.94	0.067	2.67	0.015*	0.77	0.452	-3.40	0.003**	1.39	0.181	-1.50	0.149	(+) nbHerb	(+) soilP; (-) nbDTill

nbCrop: nb of crops (5 y), nbDTill: nb of deep tillage, nbSTill: nb of surface tillage, soilP: type of tillage (5 y), Itill: tillage intensity index, nbHerb: nb of herbicides, nbFung: nb of fungicides, nbInsect: nb of insecticides, Itreat: pesticide treatment intensity index, nbOrg: nb of organic matter input (5 y), qminN: quantity of mineral nitrogen (kg N.ha⁻¹), resExp: nb of residues exportation (5 y), Ifertil: fertilization intensity index, n.s.: no significant effect.

As for cropping systems, total macrofauna density was not significantly influenced by intensity indexes (Table 2.4a). Yet, the number of orders was significantly higher under high treatment intensity in 2020, and lower under high tillage intensity in 2021 (Table 2.4b). In addition, we observed various effects of intensity indexes on macroarthropod groups in both years. An increase in tillage intensity was related to an increase in the density of Diplopoda (i.e. Polydesmida) in 2020 and of Curculionidae in 2021 and to a decrease in the density of Chilopoda in 2021 (Table 2.4a). On the other hand, an increase in pesticide treatment intensity was associated with an increased density of Araneae in 2020 and Chilopoda (i.e. Lithobiomorpha) in 2021, and with a decrease in Diplopoda (i.e. Julida) density in 2020 (Table 2.4a). Lastly, an increase in fertilization intensity had a positive effect on the density of Diplopoda (i.e. Polydesmida) and Staphylinidae in 2020, and a negative effect on the density of Curculionidae in 2020 and Chilopoda (i.e. Geophilomorpha) in 2021 (Table 2.4a). Additional effects were observed using primary practice indicators as presented in Table 2.4.

The PCA on the density of macrofauna groups showed that in 2020 macrofauna communities in CA and Conv-CA differed from communities in Conv-OA and CA-OA due to a low density of all groups (Figure 2.1a). In addition, communities were highly variable between plots within CA in 2020, and within CA-OA in 2021 (Figure 2.1b).

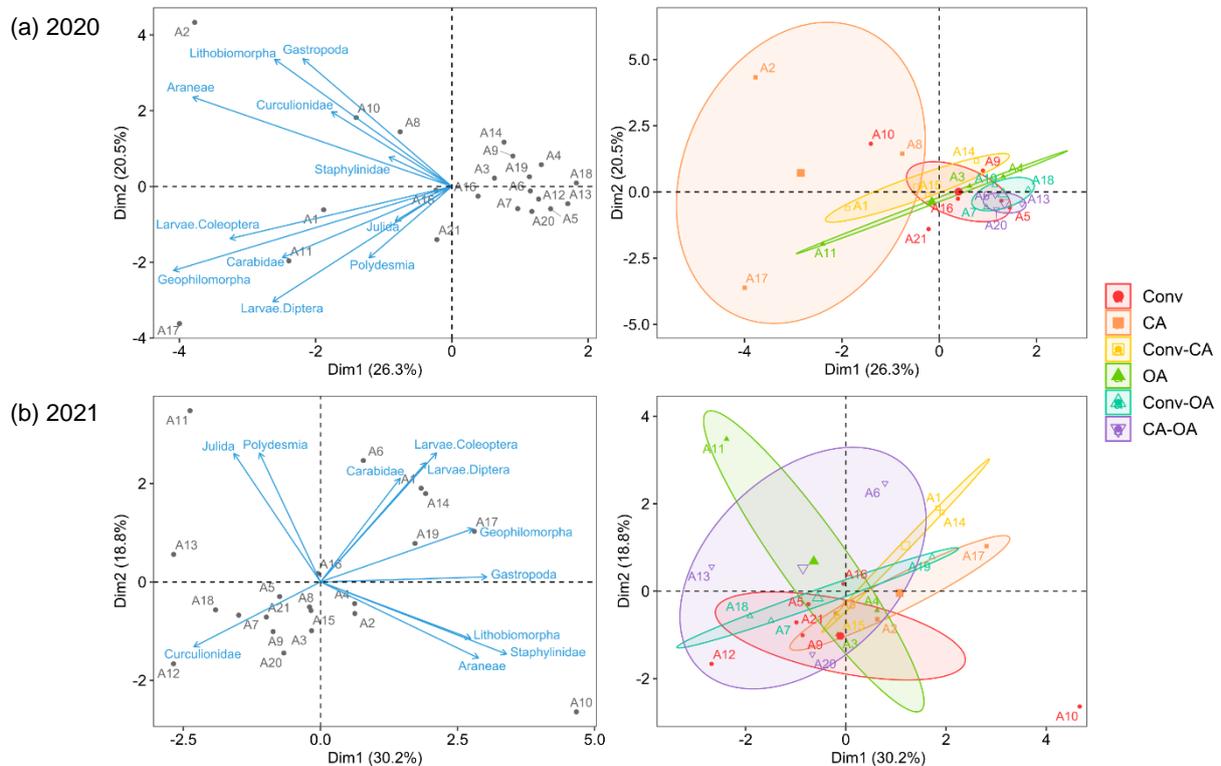


Figure 2. 1 PCA on the density of macrofauna groups (excluding earthworms) in (a) 2020 and (b) 2021. Points represent plots and ellipses are grouping plots of the same cropping system. Conv, CA and OA: conventional, conservation and organic agriculture; Conv-CA, Conv-OA and CA-OA: initial-recent system transitions.

2.3.2 Cropping system effects on earthworm density, biomass and species diversity

On average, the density of earthworms was 271 ind.m⁻² in 2020 and 265 ind.m⁻² in 2021 (Table 2.5). We observed more juveniles than adults in all systems. Earthworm total density, total biomass and Shannon index did not differ significantly between systems in 2020 (Table 2.5a and b, Figure 2.2a). In 2021, total earthworm biomass was significantly higher in Conv-CA than in Conv (Table 2.5b), and Shannon index was higher in Conv-CA than in CA-OA (Figure 2.2a). Yet, the ratio of adults/juveniles biomass was higher in OA than in Conv-OA in 2021 (Table 2.5b). Total density, biomass or diversity were not related to practice intensity (Table 2.6, Figure 2.2a).

Earthworms were mostly endogeic or epi-anecic, and one species was attributed to the intermediate ecological category (i.e. epi-endo-anecic). As almost no epigeic individuals were collected (five juveniles in A13 in 2020, one adult in A3 in 2021), they were grouped with epi-anecic for the analyses. Regarding system types, the density of epi-anecic juveniles was higher in CA than in OA in 2020, but we found no other difference neither in 2020 nor in 2021 (Table 2.5a and b). Using intensity indicators, an increase in tillage intensity was observed to be associated with a significant decrease in the density and biomass of total and epi-anecic juveniles in 2020 (Table 2.6a and b), with similar results for biomass in 2021 (Table 2.6b), and with an increase in the ratio of adults/juveniles in both years. Pesticide treatment intensity showed no significant effect. An increase in fertilization intensity had a negative effect on the density of epi-anecic adults and the biomass of total epi-anecic in 2020, and a positive effect on the density and biomass of endogeic adults in 2021. Additional effects of primary indicators are presented in Table 2.6.

Eight species of earthworms were identified each year. Species with the higher occurrence were *Allolobophora chlorotica* (Savigny, 1826), *Aporrectodea caliginosa* (Savigny, 1826) and *Aporrectodea longa* (Ude, 1885), followed by *Aporrectodea rosea* (Savigny, 1826) and *Aporrectodea icterica* (Savigny, 1826) (Table S2.3). Only one individual of *Lumbricus friendi* Cognetti, 1904 was collected in 2020 and one of *Lumbricus castaneus* (Savigny, 1826) in 2021. *A. chlorotica*, *A. caliginosa* and *A. longa* were encountered in all systems in both years. *A. rosea* was absent in almost all organic plots (except in one CA-OA plot in 2020 and one Conv-OA plot in 2021) (Table S2.3). *Lumbricus terrestris* Linnaeus, 1758 was absent from all stable systems in both years. Yet, we found no significant effect of cropping systems on the density or biomass of species (Table S2.3). In addition, the PCoA showed no differences in earthworm species community between years or cropping systems (Figure S2.1). However, the density and biomass of *A. icterica* (in 2021 only) and *A. rosea* decreased as tillage intensity increased (Table 2.7a and b). In addition, density and biomass of *A. caliginosa* and biomass of *L. terrestris* decreased as pesticide treatment intensity increased in 2020 (Table 2.7a and b). Lastly, higher fertilization intensity was related to a decrease in *A. longa* density and biomass in 2020 and to an increase in *A. rosea* in 2021 (Table 2.7a and b).

Table 2. 5 Earthworm (a) density (ind.m⁻²) and (b) biomass (g.m⁻²) in different cropping systems in 2020 and 2021. Values are means \pm standard deviations. Different lower-case letters and values in bold indicate significant differences between cropping systems according to the results of GLM with a quasi-Poisson error distribution ($P < 0.05$).

	2020						2021					
	Conv	CA	Conv-CA	OA	Conv-OA	CA-OA	Conv	CA	Conv-CA	OA	Conv-OA	CA-OA
(a) Density												
Total	252 \pm 58	284 \pm 161	343 \pm 140	148 \pm 98	340 \pm 155	281 \pm 301	206 \pm 121	196 \pm 80	402 \pm 113	212 \pm 147	404 \pm 220	233 \pm 258
Endogeic	147 \pm 86	76 \pm 56	167 \pm 111	64 \pm 56	226 \pm 183	160 \pm 204	152 \pm 142	75 \pm 58	208 \pm 53	101 \pm 86	276 \pm 88	60 \pm 61
Adults	35 \pm 42	23 \pm 3	43 \pm 42	14 \pm 20	82 \pm 86	57 \pm 94	19 \pm 13	30 \pm 20	39 \pm 25	28 \pm 13	28 \pm 16	11 \pm 18
Juveniles	112 \pm 58	53 \pm 53	124 \pm 75	50 \pm 37	144 \pm 97	103 \pm 113	133 \pm 132	44 \pm 38	169 \pm 47	73 \pm 76	247 \pm 91	50 \pm 46
Epi-aneic	46 \pm 24	176 \pm 87	110 \pm 53	27 \pm 23	112 \pm 111	107 \pm 104	29 \pm 19	105 \pm 20	107 \pm 24	34 \pm 36	117 \pm 143	37 \pm 18
Adults	5 \pm 7	18 \pm 26	7 \pm 6	7 \pm 6	28 \pm 31	14 \pm 20	4 \pm 6	7 \pm 8	12 \pm 13	9 \pm 3	7 \pm 8	5 \pm 5
Juveniles	41 \pm 19	158 \pm 68	103 \pm 52	20 \pm 17	84 \pm 81	92 \pm 109	26 \pm 22	98 \pm 27	94 \pm 20	23 \pm 31	110 \pm 136	32 \pm 14
	ab	a	ab	b	ab	ab						
Intermediate	60 \pm 97	32 \pm 51	66 \pm 73	57 \pm 85	2 \pm 3	14 \pm 25	25 \pm 39	16 \pm 19	87 \pm 71	76 \pm 119	11 \pm 14	135 \pm 234
Adults	18 \pm 24	32 \pm 51	43 \pm 46	30 \pm 39	2 \pm 3	14 \pm 25	11 \pm 17	12 \pm 13	25 \pm 22	27 \pm 33	5 \pm 9	27 \pm 46
Juveniles	42 \pm 97	0 \pm 0	23 \pm 27	27 \pm 46	0 \pm 0	0 \pm 0	14 \pm 23	4 \pm 6	62 \pm 49	50 \pm 86	5 \pm 5	108 \pm 188
Total adults	58 \pm 40	73 \pm 48	92 \pm 90	52 \pm 34	112 \pm 86	85 \pm 85	33 \pm 11	50 \pm 16	76 \pm 37	66 \pm 26	41 \pm 8	43 \pm 44
Total juveniles	195 \pm 58	212 \pm 116	251 \pm 51	96 \pm 65	228 \pm 74	196 \pm 219	173 \pm 117	146 \pm 64	325 \pm 79	146 \pm 126	363 \pm 215	190 \pm 215
Ratio ad/juv	0.3 \pm 0.2	0.3 \pm 0.1	0.3 \pm 0.3	0.6 \pm 0.1	0.4 \pm 0.3	0.4 \pm 0.4	0.2 \pm 0.1	0.4 \pm 0	0.2 \pm 0.1	1.3 \pm 1.7	0.1 \pm 0.1	0.2 \pm 0
(b) Biomass												
Total	51 \pm 22	98 \pm 58	74 \pm 28	40 \pm 4	112 \pm 55	93 \pm 79	30 \pm 9	66 \pm 20	94 \pm 6	55 \pm 17	63 \pm 47	56 \pm 54
							b	ab	a	ab	ab	ab
Endogeic	22 \pm 23	18 \pm 15	24 \pm 18	15 \pm 19	41 \pm 44	25 \pm 37	16 \pm 12	17 \pm 11	31 \pm 7	21 \pm 20	25 \pm 7	8 \pm 8
Adults	14 \pm 20	9 \pm 3	12 \pm 12	9 \pm 14	29 \pm 34	15 \pm 24	6 \pm 4	12 \pm 8	13 \pm 9	13 \pm 14	8 \pm 4	3 \pm 5
Juveniles	8 \pm 6	9 \pm 13	13 \pm 7	6 \pm 5	12 \pm 10	10 \pm 13	10 \pm 9	5 \pm 3	18 \pm 2	8 \pm 8	18 \pm 6	5 \pm 4
Epi-aneic	23 \pm 19	74 \pm 49	40 \pm 7	18 \pm 19	70 \pm 48	63 \pm 53	11 \pm 5	46 \pm 23	57 \pm 13	25 \pm 6	36 \pm 42	35 \pm 29
Adults	13 \pm 19	23 \pm 33	10 \pm 9	10 \pm 9	51 \pm 39	32 \pm 46	4 \pm 7	8 \pm 9	17 \pm 15	17 \pm 6	10 \pm 10	14 \pm 18
Juveniles	11 \pm 7	52 \pm 28	30 \pm 6	8 \pm 10	19 \pm 11	31 \pm 37	7 \pm 6	38 \pm 20	40 \pm 5	8 \pm 10	26 \pm 33	20 \pm 13
Intermediate	5 \pm 6	6 \pm 9	9 \pm 11	8 \pm 11	1 \pm 1	5 \pm 9	3 \pm 4	3 \pm 3	6 \pm 5	9 \pm 13	1 \pm 2	14 \pm 24
Adults	3 \pm 4	6 \pm 9	8 \pm 9	6 \pm 8	1 \pm 1	5 \pm 9	2 \pm 3	3 \pm 3	3 \pm 3	5 \pm 7	1 \pm 2	7 \pm 12
Juveniles	2 \pm 4	0 \pm 0	1 \pm 2	2 \pm 3	0 \pm 0	0 \pm 0	1 \pm 2	0 \pm 0	3 \pm 2	3 \pm 6	0 \pm 0	7 \pm 12
Total adults	30 \pm 19	38 \pm 29	30 \pm 27	25 \pm 4	82 \pm 49	52 \pm 51	12 \pm 7	23 \pm 1	33 \pm 13	35 \pm 10	19 \pm 9	24 \pm 27
Total juveniles	21 \pm 5	61 \pm 39	44 \pm 5	15 \pm 5	31 \pm 7	41 \pm 50	18 \pm 9	43 \pm 20	61 \pm 7	20 \pm 16	44 \pm 38	32 \pm 27
Ratio ad/juv	1.4 \pm 0.8	0.6 \pm 0.4	0.7 \pm 0.6	1.8 \pm 0.7	2.5 \pm 1.2	1.6 \pm 2.3	1.1 \pm 1.4	0.6 \pm 0.2	0.6 \pm 0.3	3 \pm 2.1	0.5 \pm 0.2	0.8 \pm 0.5
							ab	ab	ab	a	b	ab

Conv, CA and OA: conventional, conservation and organic agriculture, Conv-CA, Conv-OA and CA-OA: initial-recent system transitions, larv.: larvae.

Table 2. 6 Effect of practice intensity on earthworm (a) density (ind.m⁻²) and (b) biomass (g.m⁻²) in 2020 and 2021. t -value and P -value were obtained using GLM with a quasi-Poisson error distribution. Bold values indicate significant effects ($P < 0.05$ *, < 0.01 **, < 0.001 ***). Indicators with a significant positive (+) or negative (-) effect are reported.

	2020						2021						Indicators 2020	Indicators 2021
	Itill		Itreat		Ifertil		Itill		Itreat		Ifertil			
	t	P	t	P	t	P	t	P	t	P	t	P		
(a) Density														
Total	-1.93	0.069	0.89	0.384	-0.56	0.580	-0.53	0.604	-0.26	0.799	0.76	0.457	(-) nbSTill	n.s.
Endogeic	-0.06	0.950	-0.27	0.788	0.08	0.935	-0.19	0.856	0.32	0.756	1.70	0.106	n.s.	n.s.
Adults	-0.05	0.963	-1.15	0.263	-0.02	0.985	-0.79	0.441	-0.07	0.942	2.15	0.045*	n.s.	n.s.
Juveniles	-0.07	0.944	0.23	0.822	0.15	0.886	-0.03	0.974	0.34	0.738	1.46	0.161	n.s.	n.s.
Epi-aneic	-3.20	0.005**	0.73	0.475	-1.34	0.195	-1.44	0.167	-0.06	0.949	-0.27	0.792	(+) soilP; (-) nbDTill, nbSTill	(+) soilP
Adults	-0.42	0.677	-0.48	0.637	-2.37	0.028*	-0.88	0.392	0.57	0.573	-1.45	0.164	n.s.	(-) nbCrop
Juveniles	-3.56	0.002**	0.95	0.353	-1.16	0.261	-1.40	0.179	-0.14	0.894	-0.13	0.899	(+) soilP; (-) nbDTill, nbSTill	(+) soilP
Intermediate	-0.88	0.392	1.41	0.174	0.05	0.961	0.29	0.773	-1.01	0.327	-0.12	0.909	n.s.	(+) nbCrop; (-) nbSTill
Adults	-0.69	0.501	1.10	0.285	0.03	0.978	0.15	0.886	-0.60	0.555	-0.13	0.899	n.s.	(-) nbSTill
Juveniles	-1.04	0.311	1.14	0.267	0.08	0.936	0.35	0.732	-1.13	0.271	-0.11	0.915	n.s.	(+) nbCrop; (-) nbSTill
Total adults	-0.60	0.553	-0.36	0.723	-0.30	0.765	-0.59	0.563	-0.37	0.717	0.84	0.414	n.s.	(-) nbSTill
Total juveniles	-2.60	0.017*	1.46	0.160	-0.65	0.526	-0.48	0.639	-0.22	0.828	0.69	0.499	(-) nbDTill, nbSTill	n.s.
Ratio ad/juv	1.21	0.241	-1.96	0.065	0.28	0.783	0.94	0.360	-1.21	0.241	-0.09	0.929	n.s.	n.s.
(b) Biomass														
Total	-1.25	0.226	0.01	0.990	-0.89	0.384	-1.39	0.180	-0.01	0.993	0.25	0.802	(-) nbSTill	n.s.
Endogeic	-0.16	0.875	-0.47	0.646	0.70	0.491	-0.45	0.657	0.23	0.825	2.43	0.025*	n.s.	n.s.
Adults	0.33	0.745	-1.05	0.309	0.58	0.569	-0.59	0.564	-0.21	0.834	2.13	0.046*	n.s.	n.s.
Juveniles	-1.27	0.221	0.53	0.600	0.83	0.415	-0.12	0.906	0.51	0.615	1.63	0.119	(-) nbSTill	n.s.
Epi-aneic	-1.24	0.229	0.08	0.936	-2.12	0.048*	-1.94	0.067	0.24	0.811	-0.46	0.652	(-) nbSTill	(+) soilP
Adults	0.24	0.813	-0.55	0.592	-1.60	0.125	0.13	0.895	-0.35	0.730	-0.38	0.712	(-) qminN	n.s.
Juveniles	-2.34	0.030*	0.95	0.352	-1.68	0.109	-2.49	0.022*	0.59	0.564	-0.37	0.718	(+) soilP; (-) nbDTill, nbSTill	(+) soilP
Intermediate	-0.90	0.382	0.94	0.358	0.22	0.831	0.41	0.686	-1.26	0.225	-0.49	0.633	(-) nbSTill	(-) nbSTill
Adults	-0.69	0.496	0.66	0.515	0.20	0.843	0.30	0.768	-1.15	0.265	-0.63	0.537	(-) nbSTill	(-) nbSTill
Juveniles	-1.75	0.096	1.04	0.311	0.12	0.905	0.57	0.574	-1.42	0.171	-0.32	0.750	n.s.	n.s.
Total adults	0.21	0.836	-0.86	0.403	-0.38	0.706	-0.18	0.860	-0.75	0.464	0.58	0.571	(-) qminN	n.s.
Total juveniles	-2.80	0.011*	1.10	0.284	-1.11	0.281	-1.82	0.084	0.44	0.665	0.02	0.988	(+) soilP; (-) nbDTill, nbSTill	(+) soilP; (-) nbSTill
Ratio ad/juv	2.29	0.034*	-1.32	0.201	0.75	0.463	1.99	0.061	-1.24	0.230	0.68	0.505	(-) nbDTill, soilP	(-) soilP

nbCrop: nb of crops (5 y), nbDTill: nb of deep tillage, nbSTill: nb of surface tillage, soilP: type of tillage (5 y), Itill: tillage intensity index, nbHerb: nb of herbicides, nbFung: nb of fungicides, nbInsect: nb of insecticides, Itreat: pesticide treatment intensity index, nbOrg: nb of organic matter input (5 y), qminN: quantity of mineral nitrogen (kg N.ha⁻¹), resExp: nb of residues exportation (5 y), Ifertil: fertilization intensity index, n.s.: no significant effect.

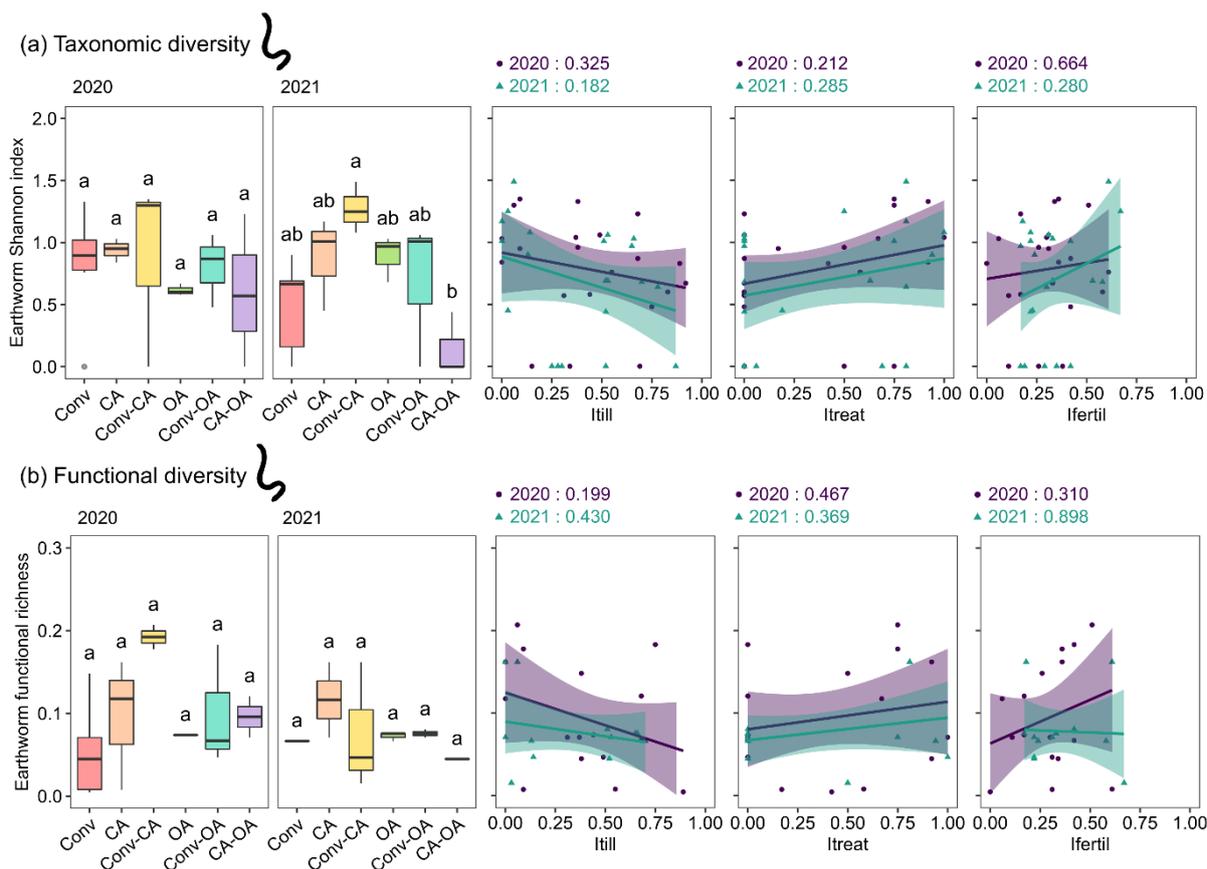


Figure 2. 2 Effect of cropping systems and practice intensity indexes on earthworm (a) taxonomic diversity (Shannon index) and (b) functional richness in 2020 and 2021. Significant differences between systems are represented with different lower-case letters according to the results of GLM with a quasi-Poisson distribution. Intensity indexes had no significant effects ($P > 0.05$).

2.3.3 Cropping system effects on earthworm functional diversity and percentage of affiliation to ecological categories

Indexes of earthworm functional diversity did not differ significantly between systems and did not respond to practice intensity, as it is reported here for the functional richness (Figure 2.2b). Furthermore, the PCA on CWM of earthworm species traits did not show different functional communities in different cropping systems (Figure S2.2). However, specific earthworm traits were influenced by cropping systems and practice intensity (Table 2.7c). In 2020, Conv-CA systems had a significantly higher proportion of earthworm species with a short body (20-50 mm) and producing small diameter cocoons (< 4 mm) than Conv-OA systems (GLM, $P < 0.05$).

In 2020, short-bodied earthworms (20-50 mm) decreased as tillage intensity increased and pesticide treatment intensity decreased (Table 2.7c). In both years, earthworms with a large body length (> 100 mm), large body mass/length ratio (> 15 g.mm⁻¹) and a rigid epithelium were disfavored under high fertilization intensity (Table 2.7c). In addition, in 2020, high fertilization

intensity favored species with medium carbon preference (20-33 mg.kg⁻¹) and had the opposite effect on species with high carbon preference (>33 mg.kg⁻¹) (Table 2.7c).

The percentage of species belonging to the three ecological categories was similar in all cropping systems (GLM, $P > 0.05$) and was not influenced by practice intensity in 2020 (Table 2.7d). However, in 2021, we observed a decrease in the representation of epigeic and anecic species and an increase in endogeic species as fertilization intensity increased (Table 2.7d), which is consistent with the observed increase in endogeic adults (Table 2.6a).

Table 2. 7 Effect of practice intensity on earthworm (a) species density (ind.m⁻²), (b) species biomass (g.m⁻²), (c) functional traits and (d) percentage of attribution to ecological categories (%) in 2020 and 2021. Functional traits and ecological categories were assessed with CWM. Only attributes of species traits that were significantly related to one of the indexes are reported. t -value and P -value were obtained using GLM with a quasi-Poisson error distribution. Bold values indicate significant effect ($P < 0.05$ *, < 0.01 **, < 0.001 ***).

	2020						2021					
	Itill		Itreat		Ifertil		Itill		Itreat		Ifertil	
	t	P	t	P	t	P	t	P	t	P	t	P
(a) Species density												
<i>A. chlorotica</i>	-0.69	0.501	1.10	0.285	0.03	0.978	0.15	0.886	-0.60	0.555	-0.13	0.899
<i>A. caliginosa</i>	1.00	0.330	-2.54	0.020*	-0.25	0.805	0.59	0.562	-1.20	0.244	0.40	0.692
<i>A. giardi</i>	-0.07	0.944	-1.08	0.292	-0.61	0.550	-1.50	0.151	0.60	0.559	-1.57	0.134
<i>A. icterica</i>	-0.80	0.434	1.72	0.102	0.97	0.344	-2.72	0.013*	1.96	0.065	1.15	0.264
<i>A. longa</i>	-0.50	0.623	-0.27	0.790	-3.11	0.006**	-0.84	0.412	0.71	0.488	-1.00	0.329
<i>A. rosea</i>	-3.66	0.002**	1.61	0.125	-0.19	0.852	-2.81	0.011*	1.31	0.206	2.24	0.037*
<i>L. terrestris</i>	0.15	0.882	-0.40	0.694	2.05	0.055	1.67	0.110	-1.92	0.070	-0.61	0.552
(b) Species biomass												
<i>A. chlorotica</i>	-0.70	0.493	0.67	0.510	0.20	0.846	0.30	0.768	-1.14	0.270	-0.63	0.534
<i>A. caliginosa</i>	1.25	0.227	-2.28	0.034*	0.22	0.826	0.66	0.516	-1.36	0.190	0.91	0.375
<i>A. giardi</i>	0.24	0.813	-0.88	0.389	-0.02	0.985	0.21	0.833	-0.21	0.834	-1.54	0.139
<i>A. icterica</i>	-1.11	0.282	1.82	0.085	1.65	0.116	-2.85	0.010*	2.05	0.055	1.22	0.239
<i>A. longa</i>	-0.15	0.886	-0.21	0.837	-3.50	0.002**	-0.11	0.913	0.06	0.950	0.07	0.940
<i>A. rosea</i>	-4.01	<0.001***	2.08	0.051	-0.35	0.733	-2.64	0.016*	1.42	0.172	1.94	0.067
<i>L. terrestris</i>	1.11	0.281	-2.21	0.040*	0.89	0.385	1.67	0.112	-1.92	0.071	-0.35	0.734
(c) Functional traits												
BL20_50	-3.16	0.005**	2.37	0.029*	-0.53	0.600	-1.29	0.214	1.25	0.227	1.81	0.087
BL>100	-0.01	0.994	0.23	0.824	-2.64	0.017*	-0.31	0.760	0.11	0.912	-2.98	0.008**
MLratio>15	0.33	0.747	-0.04	0.965	-2.77	0.013*	0.50	0.620	-0.76	0.458	-2.66	0.015*
Epi_rigid	-0.01	0.994	0.23	0.824	-2.64	0.017*	-0.31	0.760	0.11	0.912	-2.98	0.008**
Corg20_33	0.72	0.484	-0.18	0.860	2.91	0.009**	-1.26	0.224	0.09	0.926	1.13	0.274
Corg>33	-1.16	0.261	0.41	0.687	-2.25	0.037*	1.22	0.239	-0.38	0.708	-0.66	0.518
(d) Ecological categories												
%epigeic	0.08	0.940	0.61	0.547	-1.26	0.224	0.80	0.434	-0.51	0.615	-2.66	0.015*
%anecic	0.20	0.841	0.45	0.658	-0.96	0.348	-0.19	0.852	0.06	0.949	-2.23	0.038*
%endogeic	-0.19	0.853	-0.64	0.528	0.97	0.346	-0.07	0.949	0.09	0.932	2.04	0.056*

Itill: tillage intensity index, Itreat: pesticide treatment intensity index, Ifertil: fertilization intensity index, BL: body length, MLratio: mass/length ratio, Epi: epithelium type, Corg: organic carbon preferences.

2.4 Discussion

2.4.1 Comparative effects of systems and practice intensity on soil macrofauna

In this study, the total density and diversity of macrofauna were not significantly different between alternative and conventional systems, nor between stable and transitioning systems. Instead, our results confirmed that responses to agricultural management vary among macroarthropod taxa (Fuller et al., 2005; Hernández et al., 2017; Tsutsui et al., 2018) and showed variability across years. Stable conservation systems had the highest density of Chilopoda in 2020, probably as they benefited from the surface layers of residues and from the associated increase in prey number at the soil surface (Ayuke et al., 2019; Olayemi et al., 2022). In addition, both stable and recent conservation systems tended to promote the density of Coleoptera larvae compared to conventional systems. Collected larvae were mostly identified as Carabidae, suggesting that conservation systems promote Carabidae through an increase in the number and survival of larvae (Menalled et al., 2007; Henneron et al., 2015). In line with this, we observed a higher density of Carabidae in recent conservation systems than in conventional and organic systems in 2021.

Likewise, total earthworm density, biomass and diversity were similar in conventional and stable organic and conservation systems. Yet, we found a higher density of epi-anecic juveniles in conservation than in organic systems in 2020. Similarly, Pelosi et al. (2009) reported higher density and biomass of anecic and epigeic earthworms in conservation than in conventional and organic systems, but with important variability between years. In contrast with our results, previous studies also reported a higher total biomass (Hernández et al., 2017) and diversity of earthworms (Pelosi et al., 2009; Dulaurent et al., 2022) under conservation than under conventional systems. Furthermore, our results showed that earthworms may be influenced by transitional more than by stable systems. Indeed, recent conservation systems had a higher total biomass and species diversity of earthworms than conventional systems in 2021, which is consistent with the results reported for conservation systems in Pelosi et al. (2009). Furthermore, recent organic systems had a lower ratio of adults/juveniles biomass than stable organic systems in 2021, which was related with a trend for higher density and biomass of epi-anecic and endogeic juveniles. This higher proportion of juvenile earthworms in recent organic systems may be transitory and reflect changes occurring during the transition process (Irmiler, 2010). In addition, we recovered *L. terrestris* only in transitioning systems, which could suggest that this species is occurring following changes in disturbances, however this result is not confirmed by previous studies (Pelosi et al., 2015).

Tillage, pesticide treatment and fertilization intensity indexes did not reveal more effects than observed with cropping systems on overall density and diversity of macroarthropods and

earthworms. However, they allow to better characterize the effect of cropping systems on macroarthropod taxa and earthworm ecological categories, species and traits. In addition, as practices may compensate one another within cropping systems, intensity indexes are useful to better understand how cropping systems influence soil macrofauna. Tillage can affect macrofauna through direct effects, such as physical damage mostly on large species (Wardle, 1995; Chan, 2001; Pelosi et al., 2014b), and through indirect effects including the destruction of habitats (i.e. burrows, surface residues), change in soil parameters (e.g. organic matter distribution, soil water content and temperature) and predation by bird as organisms are brought to the surface during tillage (Kladivko, 2001; Roger-Estrade et al., 2010; Pelosi et al., 2017). Fertilization has also a wide range of effects on soils that can influence macrofauna. Mineral fertilizer inputs can affect soil properties causing for instance a decrease in soil pH (i.e. N fertilizers) and in soil organic carbon, and promote plant growth without being directly edible by macrofauna organisms (Birkhofer et al., 2008; de Souza and Freitas, 2018). Inversely, organic matter inputs (e.g. manure, compost) and residue retention increase trophic resources directly available for soil organisms (Ponge et al., 2013), benefit soil structuration and water and nutrient retention (de Souza et al., 2017; Olayemi et al., 2022) and provide additional microhabitats (de Souza and Freitas, 2018). Lastly, pesticide treatments are reported to have direct toxic effects on soil organisms (Pelosi et al., 2014a; Pearsons and Tooker, 2021) and can affect the availability of food source through a decrease in weed biomass (i.e. herbicides).

In our study, macroarthropods were influenced by different practices with the significance and direction of the response varying according to taxa and year. It is noteworthy that none of the effects of practice intensity were significant in both years for the same taxa. Therefore, this discussion relies on trends observed on larger groups. First, Diplopoda were the main representatives of macrofauna detritivores in our samples and we hypothesized their density to be higher under high fertilization intensity due to an increase in coarse organic matter, and lower under high tillage intensity due to the burial of organic matter. However, we found Diplopoda density to benefit from both tillage and fertilization intensity (i.e. *Polydesmida* in 2020), and to decrease under high pesticide treatment intensity (i.e. *Julida* in 2020). This suggests that Diplopoda are mostly impacted by pesticides, which is in agreement with reported detrimental effects of pesticides on macrofauna decomposers through toxic or repellent action (Domínguez et al., 2014; Pearsons and Tooker, 2021). In addition, Araneae, Chilopoda and Staphylinidae were observed to be the main predators, which we expected to decrease under high treatment intensity due to the decrease in preys (i.e. pest species). Yet, as treatment intensity increased we found a higher density of Araneae (in 2020) and of Chilopoda (i.e. *Lithobiomorpha* in 2021). In contrast, Chilopoda were negatively impacted by tillage. In line with this, no-tillage was previously reported to benefit predator macroarthropods (Stubbs et al., 2004), which is explained by a decrease in

physical disturbances and an increase in surface residues providing habitat for prey organisms (Kladivko, 2001). In addition, fertilization intensity had either a positive or negative effect depending on predator taxa. Overall, the effect of fertilization on macroarthropods seems ambiguous, which emphasizes the need for a long-term assessment of fertilization effects on soil macrofauna (de Souza et al., 2016). In addition, our results show that primary indicators of fertilization intensity (i.e. number of organic inputs, residues exportations) had different effect on soil organisms. However, these indicators only take into account a limited number of parameters, which could prevent the observation of more complex relations.

Regarding more specifically the effect of practices on earthworms, tillage intensity reportedly affects earthworm abundance and diversity with highly variable responses (Chan, 2001) and negative effect mostly on large epi-anecic species (Briones and Schmidt, 2017). In addition, the type of fertilization is reported to influence the total earthworm community, in particular with benefits of organic matter inputs (Birkhofer et al., 2008), but the distinction of this effect for different ecological categories of earthworms was rarely investigated (Betancur-Corredor et al., 2023). In contrast, pesticide treatments were reported to affect earthworms through direct effects, namely a decrease in the rate of survival and reproduction or changes in feeding behavior (Pelosi et al., 2014a).

In our study, tillage intensity affected mostly earthworms at the juvenile stage. Indeed, an increase in tillage intensity had a negative effect on the density and biomass of epi-anecic juveniles, which strongly influenced the total density of juveniles and the ratio of adults/juveniles biomass. In line with this, juvenile earthworms from all ecological groups were reported to feed more superficially than adults and to be particularly sensitive to tillage (Briones and Schmidt, 2017). However, at the species level, (i.e. adult stage), we detected a negative effect of tillage only for *A. rosea* and *A. icterica* (i.e. two endogeic species). Therefore, adults and juveniles of the same ecological category did not follow the same trend regarding tillage intensity. Furthermore, we found fertilization intensity to influence mostly adult earthworms. Indeed, an increase in fertilization intensity had a negative impact on the density of epi-anecic adult in 2020 (mostly due to a decrease in *A. longa*), and a positive effect on the density of endogeic adult in 2021 (mostly due to an increase in *A. rosea*). Lastly, we found a negative effect of pesticide treatment intensity on the density and biomass of *A. caliginosa*, and on the biomass of *L. terrestris*, whose sensitivity was observed in previous studies (Dittbrenner et al., 2011; Pelosi et al., 2014). *A. caliginosa* was notably suggested to be a good model for pesticide risk assessment (Pelosi et al., 2013).

2.4.2 Are species traits and ecological categories useful predictors of the effect of disturbances on earthworms?

The functional approach was alternatively reported to be useful (Decaëns et al., 2011; Pelosi et al., 2014b; Frazão et al., 2019) or not efficient (Hedde et al., 2012; Pelosi et al., 2016) to assess disturbance effects on earthworm communities. In our study, we found similar earthworm functional diversity and functional community composition between cropping systems. However, functional traits, considered individually, brought some insight on the effect of practices on the functional changes that may occur in earthworm communities. Contrary to our initial assumptions and as observed in previous studies (Pelosi et al., 2014), we found a higher representation of species with a small body length in recent conservation than in organic systems. Furthermore, earthworm species traits brought information on the morphological characteristics and preferences of the earthworm community depending on practice intensity. Tillage and treatment intensity were associated with species body length, and mostly acted on the presence of small species, as seen for endogeic species. Fertilization intensity influenced the presence of large species, with high mass/length ratio, rigid epithelium and a preference for high organic carbon concentration in soil, all of which are characteristics of epi-anecic species. The effect of fertilization on traits was the only effect observed both in 2020 and 2021. Contrastingly, an effect of fertilization on epi-anecic species (especially *A. longa*) was observed in 2020 only.

Overall, the low number of species collected, although consistent with the usual diversity in agricultural areas in Europe (Cluzeau et al., 2012; Rutgers et al., 2016), implies that trait attributes are related to only one or two species and follow the same trends than species density. Hence, the results reached with the functional approach could have been discussed with the sole taxonomic approach. However, it also shows that the effect of cropping systems on earthworms could be reliably studied using traits (mostly morphological traits) instead of species when the identification knowledge is missing.

Finally, ecological groups of earthworms as determined in Bottinelli et al. (2020) allowed observing differences that were hidden while considering categories commonly used in the literature. In particular, the reattribution of *A. chlorotica* to the intermediate ecological category, otherwise attributed to the endogeic category, enabled to observe the impact of fertilization on endogeic species in 2021. Indeed, *A. chlorotica* is one of the most common species encountered in cropping systems (Schmidt et al., 2001; Pelosi et al., 2015) and it seems to react to cropping practices in a different way than endogeic species. Therefore, we would like to reinforce the importance of using recently redesigned ecological groups of earthworms in future studies.

2.5 Conclusion

In this study, we show the effect of alternative cropping systems on soil macrofauna by considering the intensity of applied practice and individual macrofauna taxa rather than the entire community. Macrofauna plays an essential role in soil functioning, in particular in alternative cropping systems relying on low chemical or physical disturbances, as it supports multiple soil functions. Different taxa provide different functions with potential cascading effects on other taxa. Therefore, it is paramount to take into account the effects of cropping systems at a finer level than the whole community, especially considering macroarthropod groups (e.g. Chilopoda, Diplopoda, Coleoptera).

Furthermore, multiple methods to assess earthworm diversity such as species and functional traits are complementary and necessary to have a full overview of the impact of cropping systems. However, in our study, taxonomic and functional traits approaches of diversity yielded the same conclusions. Moreover, the updated earthworm ecological categories proved to be relevant to assess the effect of disturbances on earthworm communities, hence we would like to emphasize on the need to use these categories in forthcoming studies. Finally, the variability in macrofauna density remains high in agricultural soils. Intensity indexes are the first step for a better characterization of cropping systems, but long-term trials are now required as effects of practices on soil macrofauna can be transitory.

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———— Chapter 3 ————

Cropping systems and practice intensity affect microbial communities and bacterial traits in agricultural soils

Juliette Chassain¹, Sophie Joimel¹, Aurélie Cébron², Samuel Mondy³, Laure Vieublé Gonod¹

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¹ Université Paris-Saclay, INRAE, AgroParisTech, UMR ECOSYS, 91120, Palaiseau, France

² Université de Lorraine, CNRS, LIEC, 54000, Nancy, France

³ Université Bourgogne Franche-Comté, UMR Agroécologie, GenoSol, INRAE, 21000, Dijon, France

Abstract

Soil microorganisms are essential for the functioning of agricultural soils and may benefit from less intensive management. However, our understanding of the effect of cropping systems on the soil microbial community remains limited, in particular regarding conservation agriculture. In this study, we investigate the effects of cropping systems (conventional, organic, conservation agriculture; stable or in transition) and practice intensity (tillage, pesticide treatments, fertilization) on soil bacterial and fungal abundance, community structure, and diversity.

Soil was sampled in autumn 2020 and 2021, at 0-10 and 10-20 cm depth, over 21 plots cropped with wheat in 2020. These samples were used to characterize soil properties, soil molecular microbial biomass, and bacterial and fungal communities from the phylum to the species level using high-throughput sequencing. In addition, bacterial functional traits were assessed for the first time in cropping systems using information from the BactoTraits database.

Fungal abundance and bacterial and fungal diversity were the highest under recent organic systems and the lowest under stable conservation systems, whereas we found no effect of cropping systems on microbial biomass and bacterial abundance. In addition, bacterial diversity was positively impacted by tillage intensity, and fungal diversity was positively impacted by tillage and negatively by pesticide treatment intensity. The composition of microbial communities was influenced more by soil properties than by cropping systems, especially for bacteria. However, bacterial functional traits revealed major differences in bacterial communities between recent conservation systems and other systems. Tillage intensity influenced several bacterial traits at 10-20 cm depth (e.g. pH and temperature of growth, shape, trophic type), while pesticide treatment intensity influenced bacterial motility at 0-10 cm depth.

Overall, our results suggest that soil properties as well as cropping systems and tillage intensity are major drivers of bacterial and fungal communities in agricultural soils. Furthermore, bacterial functional traits are important indicators to understand the effect of cropping systems on the soil microbial community.

3.1 Introduction

The sustainable use of agricultural soils is crucial in order to maintain biodiversity, soil functioning and consequently crop production. Soil microorganisms represent a large part of agricultural soil biodiversity. They are key drivers of several soil processes and functions such as organic matter turnover (Swift et al., 1979), nutrient mineralization (Schloter et al., 2018) and soil structural stability (Beare et al., 1997; Six et al., 2002), hence driving soil physical and chemical fertility and allowing plant uptake and growth (Schloter et al., 2018). Their diversity and community composition are mainly driven by soil properties such as pH (Fierer and Jackson, 2006; Bahram et al., 2018; Karimi et al., 2018), texture (Johnson et al., 2003) and carbon (C) and nitrogen (N) content (Delgado-Baquerizo and Eldridge, 2019). In addition, microbial communities are closely related to the type of crop growing on the field (Johnson et al., 2003). However, cropping intensity and the associated soil disturbances are recognized as a main factor of influence on microbial communities (Karimi et al., 2018). Indeed, agricultural practices may affect soil physical, chemical and biological characteristics with cascading effects on soil microorganisms.

Intensive systems rely on practices having strong effects on soils, such as intensive tillage, pesticide treatments and use of mineral fertilizers. Therefore, crop production in conventional systems draws mostly on artificial inputs and less on the functions that could be provided by a dense and diverse microbial community. Alternative systems, such as organic and conservation agriculture, were developed in order to rely on less intensive practices fostering soil microorganisms and associated services. However, few studies compared the effect of alternative systems on soil microorganisms (Christel et al., 2021). Organic systems were reported to increase microbial biomass (Fraser et al., 1988; Scow et al., 1994), bacterial and fungal abundance (Fraser et al., 1988; Hole et al., 2005; Lori et al., 2017) and richness (Hartmann et al., 2015; Lupatini et al., 2017), and to present soil microbial community structures that differ from conventional systems (Hartmann et al., 2015). Conservation systems - defined by no-tillage, soil cover and diversified crop rotation - were observed to show higher soil microbial biomass (Govaerts et al., 2007; Sapkota et al., 2012; Palm et al., 2014), bacterial and fungal abundance (Henneron et al., 2015) and different microbial community structures (Romero-Salas et al., 2021) compared to conventional systems. Yet, other studies found contradictory or no effect of organic (Hole et al., 2005) and conservation systems (Scopel et al., 2013) on soil microorganisms, in particular when considering microbial biomass.

In addition, previous studies investigated the effects of transition from conventional to organic systems and found a gradual increase in fungal abundance and microbial biomass (Santos et al., 2012; Coller et al., 2022). However, we found no study focusing on the effects of the

transition to conservation systems, nor on the transition from conservation to organic systems, with more research centered on the transition to no-tillage (Stubbs et al., 2004; Zhang et al., 2022).

Bacterial and fungal abundance and diversity were reported to be influenced by cropping practices at short- and long-term, in particular by tillage (Wardle, 1995), pesticide treatments (Bünemann et al., 2006), fertilization (Geisseler and Scow, 2014; de Souza and Freitas, 2018; Tosi et al., 2021) and crop rotation (Sadet-Bourgeteau et al., 2018). Tillage was demonstrated to have large impacts on soil microorganisms (Le Guillou et al., 2019; Kraut-Cohen et al., 2020), especially because it affects numerous soil properties causing an increase in temperature, a decrease in moisture and changes in the structure and in the distribution of organic matter in soil (Pires et al., 2017), as well as increased exposure to predation. Tillage was suggested to decrease bacterial diversity (Le Guillou et al., 2019). However, direct effects of tillage were also observed on fungi as mixing of soil breaks their hyphae, thus impeding their growth (Beare et al., 1993; Stubbs et al., 2004). Conversely, no-tillage was demonstrated to enable a higher soil microbial biomass than conventional tillage (Balota et al., 2003; Lienhard et al., 2013; Dunn et al., 2021) and to drive changes in the soil microbial community structure (Kraut-Cohen et al., 2020). In addition, it is commonly acknowledged that no-till systems tend to be dominated by fungi while conventional systems tend to be dominated by bacteria (Six et al., 2002; Palm et al., 2014), depending on the depth at which soil is sampled (Wardle, 1995).

Fertilization was also demonstrated to be a main factor of influence for soil microorganisms (Hole et al., 2005). Repeated applications of mineral fertilizers were reported to increase microbial biomass (Geisseler and Scow, 2014) and to decrease microbial richness while increasing evenness (Hartmann et al., 2015). Positive effects of mineral fertilizers on microbial biomass are mostly observed on the long-term due to an increase in crop production and associated higher organic matter from plants in soil. However, mineral fertilizers also affect soil properties (e.g. pH) and create a lower dependence of plants on microorganisms due to a high soil N and P content. Consequently, negative effects of mineral fertilizers are reported on total microorganisms (Treseder, 2008; Fierer et al., 2012; Zhang et al., 2015) and on more specific microorganisms such as arbuscular mycorrhizal fungi or N₂-fixing bacteria (Clermont-Dauphin et al., 2014; de Graaff et al., 2019). In comparison, organic matter inputs bring additional trophic resources and may influence soil properties differently. They benefit bacterial and fungal abundance (Treonis et al., 2010), bacterial diversity (Rachwał et al., 2021) and induce changes in the bacterial and fungal communities (Lejon et al., 2007). However, the durability and intensity of these effects vary according to the characteristics of the applied organic matter (Vieublé-Gonod et al., 2009).

Pesticide treatments are considered as a second order factor as they have inconsistent effects on microorganisms (Hartmann et al., 2015). Negative effects were mostly reported for fungicides

and insecticides (Beare et al., 1993; Bünemann et al., 2006). Inversely, herbicide applications may have a positive influence on microorganism's abundance, both due to an increase in dead weed biomass at the soil surface providing more organic matter, and as an energy source for some species (Neher, 1995).

Thus, the effect of cropping systems and practices on soil microorganisms is complex and a large part of the variability in soil microbial communities in agricultural areas remains unexplained (Johnson et al., 2003). Most studies on soil microorganisms were conducted in controlled conditions or in experimental sites (Caruso and Bardgett, 2021; Rachwał et al., 2021), which do not represent the diversity of agricultural practices applied by farmers. In addition, few studies investigated the comparative effect of alternative systems and systems in transition toward these alternatives on microorganisms (Bill et al., 2021; Dunn et al., 2021). However, it is necessary to understand the influence of existing cropping systems and practices on soil properties and microbial communities in order to guarantee the sustainability of applied practices.

The development of new molecular techniques since the early 2000s has led to major advances in our knowledge of microbial ecology, in terms of abundance and taxonomic and functional microbial diversity. These techniques make it possible to avoid the use of time-consuming classical microbiological methods and to access a much larger fraction of microorganisms. Furthermore, in the recent years, the development and increasing use of the functional traits approach in several fields of ecology, such as plant (e.g. Lavorel et al., 2011) and invertebrate ecology (e.g. Pey et al., 2014), have led to a growing interest for the use of functional traits in soil microbial ecology (Yang, 2021). Functional traits indeed have the potential to further our understanding of microbial community composition, adaptation and functional role under disturbed environments (Cébron et al., 2021). This approach has been well developed for fungal communities (Crowther et al., 2014; Nguyen et al., 2016; Zanne et al., 2020), but its implementation was more complex regarding bacteria and archaea (Lajoie and Kembel, 2019). Conceptual frameworks on bacterial traits (Romillac and Santorufo, 2021), and user-friendly databases, such as BactoTraits (Cébron et al., 2021), were only very recently developed and bring additional perspectives to investigate the relation between anthropic disturbances and bacterial traits within soil communities. For now, BactoTraits has only been used in one study over contaminated soil (Cébron et al., 2022), and new studies are required to assess the response of bacterial functional traits to different cropping intensities.

In this study, we assessed the effect of alternative cropping systems, stable or in transition, and of practice intensity and soil properties on the soil bacterial and fungal communities. In addition, we investigated for the first time the effect of cropping systems and practices on the bacterial functional community using the BactoTraits database. We hypothesized that soil

microbial biomass, bacterial and fungal abundance vary according to the depth of sampling, especially in conservation systems due to the concentration of organic matter at the surface. Microbial abundance and diversity may increase in organic systems due to the mixing of organic matter and the absence of pesticide inputs. In addition, we expected soil microbial communities to be influenced by tillage and fertilization intensities and in a lesser extent by pesticide treatment intensity.

3.2 Materials and methods

3.2.1 Study sites and agricultural practices

The study was conducted in autumn 2020 and 2021 over 21 plots owned by farmers and located in the Paris basin, France. Climate is temperate with mean annual precipitations of 600 to 700 mm and a mean annual temperature of 11°C (cf. General introduction for more details).

Plots were cultivated with winter wheat in 2020-2021 and with different crops or cover crop in 2021-2022 (Table 3.1). Soils were silty or clayed, with a pH ranging from 5.5 to 8.2 and a bulk density varying between 1.1 and 1.5 g.cm⁻³ at 0-20 cm depth (Table 3.1).

Table 3. 1 Location, crop, cropping system and soil properties of the 21 plots of the study. Year since conversion corresponds to the number of years since conversion to the current system in 2020. Clay, silt, sand and pH are the average of values obtained for samples at 0-10 and 10-20 cm depth.

Plot	Region	Crop 2020	Crop 2021	System	Age	Clay	Silt	Sand	pH	SOM ₀₋₁₀	SOM ₁₀₋₂₀	BD ₀₋₁₀	BD ₁₀₋₂₀
						g.kg ⁻¹	g.kg ⁻¹	g.kg ⁻¹		g.kg ⁻¹	g.kg ⁻¹	g.kg ⁻¹	g.cm ⁻³
A1	Eure-et-Loir	wheat	wheat	Conv-CA	3	155	475	54	6.9	19	15	1.3	1.4
A2	Essonne	wheat	cover	CA	17	210	383	119	6.5	34	19	1.3	1.5
A3	Essonne	wheat	wheat	OA	20	157	414	194	6.5	18	19	1.4	1.5
A4	Yvelines	wheat	bare	OA	19	433	240	91	7.9	34	33	1.1	1.2
A5	Eure-et-Loir	wheat	barley	Conv	20	210	448	52	6.5	25	19	1.3	1.5
A6	Essonne	wheat	bare	CA-OA	2	279	377	32	6.6	21	21	1.3	1.4
A7	Yvelines	wheat	rye	Conv-OA	2	174	454	63	6.7	22	23	1.2	1.3
A8	Essonne	wheat	mustard	CA	7	193	365	183	5.5	24	17	1.5	1.5
A9	Yvelines	wheat	rapeseed	Conv	20	205	404	188	6.8	18	18	1.3	1.4
A10	Eure-et-Loir	wheat	alfalfa	Conv	20	221	453	49	7.1	21	20	1.3	1.3
A11	Yvelines	wheat	cover	OA	20	256	351	117	7.4	27	29	1.2	1.2
A12	Yvelines	wheat	cover	Conv	20	223	350	165	7.3	23	22	1.3	1.3
A13	Yvelines	wheat	rye, lentil	CA-OA	2	250	411	73	6.9	33	27	1.2	1.4
A14	Yvelines	wheat	cover	Conv-CA	3	180	383	121	6.8	32	25	1.2	1.1
A15	Yvelines	wheat	wheat	Conv-CA	3	194	394	90	6.8	28	24	1.4	1.5
A16	Essonne	wheat	mustard	Conv	20	293	337	67	8.1	24	21	1.4	1.5
A17	Yvelines	wheat	rapeseed	CA	10	228	350	198	8.1	36	31	1.2	1.4
A18	Yvelines	wheat	bare	Conv-OA	3	195	454	69	6.9	24	25	1.2	1.4
A19	Yvelines	wheat	clover	Conv-OA	2	280	266	261	7.7	24	27	1.3	1.3
A20	Eure-et-Loir	wheat	cover	CA-OA	2	202	407	51	7.3	17	21	1.4	1.4
A21	Eure-et-Loir	wheat	barley	Conv	20	336	226	244	8.0	33	28	1.2	1.3

Conv: conventional agriculture, CA: conservation agriculture, OA: organic agriculture, Conv-CA: transition from conventional to conservation (≤ 3 years), Conv-OA: transition from conventional to organic (≤ 3 years), CA-OA: transition from conservation to organic (≤ 3 years), SOM₀₋₁₀ and SOM₁₀₋₂₀: soil organic matter at 0-10 and 10-20 cm depth respectively, BD₀₋₁₀ and BD₁₀₋₂₀: bulk density at 0-10 and 10-20 cm depth respectively.

The 21 plots were selected to constitute six groups of cropping systems, namely systems under stable (≥ 7 years) conventional (Conv, $n = 6$ plots), organic (OA, $n = 3$) or conservation agriculture (CA, $n = 3$), or in transition (≤ 3 years) from conventional to organic (Conv-OA, $n = 3$), conventional to conservation (Conv-CA, $n = 3$) or conservation to organic agriculture (CA-OA, $n = 3$) (Table 3.1).

Primary indicators and composite indexes were selected to represent tillage, pesticide treatment, fertilization and crop rotation practices for each plot following Büchi et al. (2019) (Table 3.2). Composite indexes of tillage intensity (Itill) and fertilization intensity (Ifertil) were computed by an additive combination of primary indicators, and the composite index of pesticide treatment intensity (Itreat) was calculated as the normalized value of the total number of applied treatments (cf. General introduction). The mean values of primary indicators and composite indexes obtained for the six cropping systems in 2020 and in 2021 are reported in Table 3.2.

Table 3. 2 Primary indicators and composite indexes of practice intensity used to characterize cropping systems. Values are presented for 2020 and 2021. Composite indexes are given in bold. Primary indicators were computed for practices occurring in one year or in five years for nbCrop, soilP, nbOrg, resExp.

Name	Description	Mean value in cropping system (2020 ; 2021)					
		Conv	CA	Conv-CA	OA	Conv-OA	CA-OA
nbCrop	Crops in the rotation	3.2 ; 3.5	3.3 ; 3.7	3.7 ; 3.7	3.7 ; 3.7	3 ; 3.7	3.3 ; 3.7
nbDTill	Deep tillage interventions (> 15 cm)	0.7 ; 0.7	0 ; 0	0 ; 0	1.7 ; 1.3	1.7 ; 1.3	1 ; 0.3
nbSTill	Surface tillage interventions (< 15 cm)	5.3 ; 4	0.7 ; 0	0.3 ; 0.3	6.3 ; 7.7	3.7 ; 7	7 ; 8.3
soilP	Type of tillage (plough = 0.5, no-till = 5)	2 ; 2	4.9 ; 4.9	3.8 ; 4.1	1.3 ; 1.3	1.5 ; 1.5	2.3 ; 2.3
nbHerb	Herbicide treatment applications	5.2 ; 7.8	6.3 ; 6	5.3 ; 6.3	0 ; 0	0 ; 0	0 ; 0
nbFung	Fungicide treatment applications	0.8 ; 1.8	0.3 ; 1.7	1 ; 3.3	0 ; 0	0 ; 0	0 ; 0
nbInsect	Insecticide treatment applications	1.3 ; 0.8	0 ; 0.7	2.3 ; 2	0 ; 0	0 ; 0	0 ; 0
nbOrg	Organic matter inputs	0.8 ; 0.8	0.7 ; 0.7	1 ; 1	5 ; 5	3.3 ; 3.3	2.3 ; 3
qminN	Mineral N fertilizers inputs (kg N.ha ⁻¹)	129 ; 198	109 ; 130	69 ; 187	0 ; 0	0 ; 0	0 ; 0
resExp	Residues exportation	0.3 ; 0.3	0 ; 0	2.3 ; 2.3	1 ; 1	2.3 ; 2.3	0 ; 0.3
Itill	Tillage intensity	0.5 ; 0.4	0 ; 0	0.1 ; 0.1	0.7 ; 0.7	0.6 ; 0.6	0.6 ; 0.4
Itreat	Pesticide treatment intensity	0.7 ; 0.8	0.6 ; 0.6	0.8 ; 0.8	0 ; 0	0 ; 0	0 ; 0
Ifertil	Fertilization intensity	0.3 ; 0.3	0.2 ; 0.2	0.4 ; 0.5	0.4 ; 0.4	0.4 ; 0.4	0.1 ; 0.2

Conv, CA and OA: conventional, conservation and organic agriculture, Conv-CA, Conv-OA, CA-OA: initial-recent system transitions.

3.2.2 Soil sampling

The soils were sampled in autumn, from October 19th to December 2nd in 2020 and from October 25th to November 22nd in 2021, at minimum one week after sowing to allow soil to partially recover after tillage and other mechanical operations. Three replicates were defined on each plot. They were spaced by 25 m and located 25 m apart from the field margins and outside of tractor traffic tracks.

Soil samples were collected at 0-10 and 10-20 cm depth in order to account for differences in the soil structure and in the soil organic matter location in different cropping systems. Composite soil samples were collected on each replicate by mixing eight soil cores obtained with an auger. All samples were collected between crop rows. In the laboratory, soil was kept at 4°C right after sampling and a subsample was dried (105°C, 48h) to assess soil humidity.

In 2020, a fraction of sampled soils was sieved at 4 mm, air dried and the main soil characteristics were analyzed by the INRAE Laboratory of Arras. Soil cores (10 cm in diameter, 10 cm high) were collected at 0-10 cm and 10-20 cm depth, dried (105°C, 48h) and weighed to assess soil bulk density.

In 2020 and 2021, soil samples were sieved at 4 mm and about 100 g of sieved soil was stored in 30 ml vials kept at -30°C. The sieved soils were then lyophilized and kept at -30°C for conservation and microbial analyses at the GenoSol platform (INRAE, Dijon, France).

3.2.3 Molecular microbial biomass and bacteria and fungi abundances

Microbial DNA was extracted from 1g (dry weight) of soil following the protocol of Plassart et al. (2012) at the GenoSol platform (INRAE, Dijon, France). Extraction was performed by a mechanical lysis (90s, 4 m.s⁻¹) using FastPrep® followed by a chemical lysis using a buffer containing EDTA, Tris, NaCl and SDS and water-bath set at 70°C for 30 min.

The concentration of crude DNA (i.e. soil molecular microbial biomass) was determined by electrophoresis using 1% agarose gel and serial dilutions of calf thymus DNA (DNA Quantitation Kit, Fluorescence Assay, SIGMA-MERCK) as standard, and quantified using Smart Ladder (Eurogentec®) as a DNA molecular size marker. After migration, the gel was soaked in an ethidium bromide (BET) bath, rinsed, and its image was captured under UV lamp (Uvidoc HD5). Images were analyzed with ImageQuANT software (Molecular Dynamics, Evry, France). DNA was purified using a NucleoSpin Soil kit (Macherey-Nagel) and quantified by a fluorometric assay using QuantiFluor® (Promega) and Infinite Pro 200 (Tecan) plate reader.

Bacterial and fungal abundances were estimated by real-time quantitative polymerase chain reaction (qPCR) of the 16S and 18S rRNA genes. Bacterial 16S rRNA genes were amplified from 2 ng of DNA and using primers 515R and 341F under the following conditions: 15min at 95°C, 35 cycles comprising 15s at 95°C, 30s at 60°C, 30s at 72°C and 20s at 80°C, and then 15s at 95°C, 15s at 60°C and 15s at 95°C. Fungal 18S rRNA genes were amplified from 2 ng of DNA and using primers FR1 and FF390 under the following conditions: 10min at 95°C, 35 cycles comprising 15s at 95°C, 30s at 50°C and 1min at 72°C, and then 15s at 95°C, 15s at 60°C and 15s at 95°C. In both cases, DNA quantity was measured with SYBRGreen® mix (Eurogentec) and StepOne Plus 96 (Applied Biosystems®) thermal cycler apparatus.

The ratio fungi/bacteria (F/B ratio) was estimated using the ratio of 18S/16S rRNA gene copies.

3.2.4 DNA sequencing - Bacterial and fungal diversity

Microbial diversity was estimated by sequencing bacterial 16S rRNA gene and fungal Internal Transcribed Spacer 2 (ITS 2). Bacterial 16S rRNA fragments were amplified by a first PCR conducted on 5 ng DNA with primers F479 (5'-CAGCMGCYGCNGTAANAC-3') and R888 (5'-CCGYCAATTCMTTTRAGT-3') under the following conditions: 2min at 94°C, 35 cycles of 30s at 94°C, 30s at 52°C and 1 min at 72°C, and 7 min at 72°C. Fungal ITS 2 fragments were amplified by a first PCR conducted on 10 ng DNA with primers ITS86-F (5'-GTGAATCATCGAATCTTTGAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') under the following conditions: 2min at 95°C, 35 cycles of 30s at 95°C, 30s at 55°C and 1 min at 72°C, and 10 min at 72°C. All PCR products were purified using magnetic beads of the Pronex Promega kit and quantified by a fluorometric assay using QuantiFluor.

Multiplex PCR was performed on 7.5 ng DNA of purified PCR products for bacterial 16S with primers Primer_P5 and Primer_P7. Conditions were the same than during the first PCR but with seven cycles only. PCR product fragments were purified using MinElute kit (Qiagen) and quantified as above. Similarly, multiplex PCR was performed on 10 ng DNA of purified PCR for fungal ITS 2, with primers Primer_P5 and Primer_P7 under the following conditions: 1min at 94°C, 12 cycles of 1min at 94°C, 1min at 65°C and 1min at 72°C, and 10min at 72°C.

Sequencing of 16S rRNA and ITS 2 fragments was performed by the GeT-PlaGe platform (INRAE, Castanet-Tolosan, France).

3.2.5 Bioinformatic analyses

The data were processed using the FROGS Pipeline version 4.0.1 (Escudié et al., 2018; Bernard et al., 2021) on the Genotoul-Sigenae Galaxy servers. The pipeline is described in Figure S3.1. The reads were merged using flash. The extended sequences were trimmed according to their size (from 300 to 800 for ITS and 300 to 500 for 16S). After the trimming step, sequences were clustered using Swarm (aggregation distance 1). Chimera removal and filtering on low abundance OTU (OTU below frequency of 5e-05) were removed for subsequent analysis. The OTU were assigned using the FROGS affiliation step (the SILVA 123 database for 16S and the UNITE 7.0 for ITS2).

The sequences were clustered by operational taxonomic units (OTU). The taxonomy of each OTU was determined at the phylum, class, order, family, genus and species levels. The data were sorted to keep only 16S sequences attributed to bacteria (excluding sequences attributed to archaea) and ITS 2 sequences attributed to fungi (excluding sequences attributed to plants, protists and unidentified domains).

Bacterial and fungal OTU datasets were rarefied to the lowest number of sequences per sample (7826 and 11393 sequences per sample for bacteria and fungi respectively) using the `rrarefy` function from R *vegan* package (Oksanen et al., 2022). Richness, Shannon index and Pielou's evenness index of bacterial and fungal OTU diversity were calculated at the plot level. Additional analyses were performed at higher taxonomic levels (phylum, class) that were suggested to be suitable to assess environmental drivers of microbial communities (Karimi et al., 2018).

3.2.6 Bacterial traits assessment

Bacterial functional traits were investigated in all samples to assess the effect of cropping systems and practice intensity on the functional community of bacteria. A total of 19 bacterial traits were attributed to our bacterial OTU using the BactoTraits database (Cébron et al., 2021). Traits were either morphological, physiological or genomic and could be related to bacterial functions or responses to environmental conditions (Cébron et al., 2021). Several bacterial traits could be related to resistance to desiccation such as gram type (Schimel et al., 2007) or pigmentation (Wada et al., 2013), and to nutrient access such as shape (Young, 2006) or trophic type (Thies and Grossman, 2006). In addition, resistance to disturbances was associated with traits such as sporulation ability (Prosser et al., 2007), motility (Alexandre et al., 2004) or the Guanine-Cytosine percent (%GC) of the genome (Nishida, 2012).

The trait profiles of bacterial OTU were obtained by attributing a relative proportion of each trait attributes to each OTU based on their taxonomic affiliation. The default rank of taxonomic affiliation used for the attribution of trait profiles was the genus. However, a higher taxonomic rank (family, order, class or phylum) was used whenever the information was not available in the database.

3.2.7 Statistical analyses

Sub-samples were pooled by plots by averaging the values of the three replicates, as replicates of the same plot are supposed to be more similar than samples of different plots (Johnson et al., 2003). Before analysis, the data were tested for normality and homogeneity of variances using a Shapiro-Wilk test ($\alpha > 0.05$) and a Bartlett test ($\alpha > 0.05$).

The relations between soil properties and cropping systems were assessed by performing a principal component analysis (PCA) on all soil properties at 0-10 and 10-20 cm depth. Differences in specific soil properties were assessed with an analysis of variance (ANOVA) followed by a Tukey HSD post-hoc test.

All microbial parameters were investigated at 0-10 and 10-20 cm depth in 2020 and 2021, namely soil microbial biomass, bacterial and fungal abundance (i.e. number of 16S and 18S copies.g soil⁻¹), bacterial and fungal OTU diversity (i.e. richness, Shannon index, Pielou's evenness index), relative abundance of bacterial and fungal phyla and bacterial trait values. As normality and homogeneity of variance were not met, differences in microbial parameters regarding cropping systems and practice intensity were assessed using generalized linear models (GLM) with a quasi-Poisson family and an identity link to account for overdispersion of data. GLM were followed by Tukey HSD post-hoc tests when comparing different cropping systems.

Dissimilarities in bacterial and fungal OTU communities in different cropping systems were assessed by performing a principal coordinates analysis (PCoA) based on Bray-Curtis distance, followed by a permutational analysis of similarities (ANOSIM) to test for significant differences in composition. Relations between soil environmental parameters and bacterial or fungal community composition at the phylum level were investigated with a redundancy analysis (RDA), followed by an ANOVA to test for its significance and for the significance of the axes. Only the soil variables that contributed the most to the RDA and that were not highly correlated (e.g. total carbon and organic matter content) were selected. However, we added organic matter content to the RDA on fungal phyla, even if no significant correlation was reported. A bidirectional hierarchical clustering of the main bacterial and fungal classes at each plot was performed using Euclidean distance. A PCA was performed on all bacterial traits to assess for differences in the functional communities between cropping systems.

All analyses were performed using R software version 3.6.3 (R Development Core Team 2020) and the *stats* (R Development Core Team 2020), *vegan* (Oksanen et al., 2022), *ade4* (Dray and Dufour, 2007), *FD* (Laliberté et al., 2022), *multcomp* (Hothorn et al., 2022) and *phreatmap* (Kolde, 2019) packages.

3.3 Results

3.3.1 Soil properties

Soil properties are presented for each plot at 0-10 and 10-20 cm depth (cf. General introduction, Tables I.5 and I.6). As shown by the PCA, soils of CA plots differed from all the other systems at 0-10 cm depth but not at 10-20 cm depth, and all CA and Conv-CA plots differed from Conv plots at both depths (cf. General introduction, Figure I.12).

Individual soil properties did not differ significantly between cropping systems, except for a higher amount of K and Mg (ANOVA, $P < 0.05$) in OA than in Conv-CA systems at 10-20 cm depth. Soil organic matter (SOM) content tended to be higher at 0-10 cm than at 10-20 cm depth in CA

(on average 31.30 and 22.41 g.kg⁻¹) but not in Conv-CA (26.52 and 21.49 g.kg⁻¹) nor in other systems. Inversely, SOM was the most homogeneous between the two depths in OA (26.31 and 26.97 g.kg⁻¹). In addition, bulk density was the highest in CA systems at both depth (1.33 and 1.43), even if no significant difference was observed with the other systems. Furthermore, among other effects, as tillage intensity (Itill) increased the C/N decreased at 0-10 cm depth, and K, Mg, Na, and P increased at 10-20 cm depth (cf. General introduction, Table I.7). An increase in the number of organic matter inputs over 5 years (nbOrg) was associated to an increase in CEC, SOM, Fe, K, Mg and Na at 10-20 cm depth (cf. General introduction, Table I.7). Lastly, compared to all the other plots, A8 plot had a rather low pH (5.5 and 5.6 at 0-10 and 10-20 cm), A4 plot had a high clay content (426 and 439 g.kg⁻¹ at 0-10 and 10-20 cm) and A17 plot had a high quantity of carbonates (143 and 144 g.kg⁻¹ at 0-10 and 10-20 cm).

3.3.2 Microbial biomass

Molecular microbial biomass represented on average 25.26 (14.74-42.69) µg DNA.g⁻¹ soil in 2020 and 28.54 (19.09 - 56.74) µg DNA.g⁻¹ soil in 2021 at 0-10 cm depth, and 24.17 (13.02 - 42.20) µg DNA.g⁻¹ soil in 2020 and 25.16 (17.10 - 51.73) µg DNA.g⁻¹ soil in 2021 at 10-20 cm depth. We found no significant difference in microbial biomass between systems (Table 3.3a) and no significant effect of practice intensity (Table 3.4a) nor of crop type (GLM, $P > 0.05$). However, an increase in the number of organic matter inputs over 5 years increased microbial biomass at 10-20 cm depth (Table 3.4a).

3.3.3 Bacterial and fungal abundance

The F/B ratio was no significantly different between systems at 0-10 cm depth (Table 3.3b), but it was lower in Conv-CA than in OA and Conv-OA at 10-20 cm depth in 2020 (Table 3.3b) in association with a lower abundance of fungi in Conv-CA (Table 3.3d). Bacterial abundance at 0-10 and 10-20 cm depth and fungal abundance at 0-10 cm depth were similar in all systems (Table 3.3c and d). Crop type in 2021 did not significantly influence F/B ratio nor bacterial or fungal abundance (GLM, $P > 0.05$).

In addition, the F/B ratio at 10-20 cm depth was significantly higher under higher tillage intensity in both years and under lower pesticide treatment intensity in 2020 (Table 3.4b), in relation to the effect of tillage and pesticide treatment intensities on fungal abundance (Table 3.4d). Fungal abundance at 0-10 cm and bacterial abundance at all depths were not significantly influenced by practice intensity (Table 3.4c and d). We observed no significant effect of fertilization intensity (Table 3.4).

Table 3. 3 Effect of cropping systems on (a) molecular microbial biomass ($\mu\text{g DNA.g}^{-1}$ soil), (b) ratio fungi/bacteria, (c) bacterial abundance (10^9 16S copies.g $^{-1}$ soil) and diversity and (d) fungal abundance (10^8 18S copies.g $^{-1}$ soil) and diversity at 0-10 and 10-20 cm depths in 2020 and 2021. Values are means \pm standard deviations. Richness, Shannon and Evenness indexes are based on the number of bacterial and fungal OTU. Different lower-case letters indicate significant differences between cropping systems within a year according to a GLM with a quasi-Poisson error distribution ($P < 0.05$).

	Depth	2020						2021					
		Conv	CA	Conv-CA	OA	Conv-OA	CA-OA	Conv	CA	Conv-CA	OA	Conv-OA	CA-OA
(a) Biomass	0-10	24 \pm 9	25 \pm 6	26 \pm 4	31 \pm 11	22 \pm 4	24 \pm 8	24 \pm 3	28 \pm 6	36 \pm 4	34 \pm 20	26 \pm 6	27 \pm 10
	10-20	24 \pm 8	16 \pm 3	24 \pm 5	31 \pm 11	26 \pm 9	23 \pm 1	23 \pm 6	23 \pm 4	26 \pm 4	34 \pm 17	23 \pm 8	24 \pm 4
(b) Ratio F/B	0-10	0.05 \pm 0.02	0.05 \pm 0.02	0.03 \pm 0.01	0.04 \pm 0.01	0.05 \pm 0.00	0.04 \pm 0.00	0.05 \pm 0.02					
	10-20	0.03 \pm 0.01	0.03 \pm 0.01	0.02 \pm 0.00	0.04 \pm 0.02	0.05 \pm 0.01	0.04 \pm 0.01	0.03 \pm 0.01	0.03 \pm 0.00	0.02 \pm 0.01	0.03 \pm 0.01	0.04 \pm 0.01	0.03 \pm 0.01
(c) Bacteria	Abundance	12 \pm 4	11 \pm 3	11 \pm 1	14 \pm 3	10 \pm 2	12 \pm 3	11 \pm 1	15 \pm 2	16 \pm 1	16 \pm 6	11 \pm 2	14 \pm 4
	Richness	1336 \pm 100	1213 \pm 183	1464 \pm 8	1365 \pm 90	1415 \pm 41	1448 \pm 47	1359 \pm 126	1215 \pm 153	1467 \pm 5	1349 \pm 113	1462 \pm 32	1422 \pm 99
Shannon	0-10	6.3 \pm 0.1	6.1 \pm 0.2	6.4 \pm 0.0	6.3 \pm 0.1	6.5 \pm 0.1	6.4 \pm 0.1	6.2 \pm 0.3	6.1 \pm 0.2	6.3 \pm 0.0	6.2 \pm 0.2	6.4 \pm 0.1	6.3 \pm 0.2
	10-20	6.3 \pm 0.1	5.8 \pm 0.5	6.3 \pm 0.1	6.4 \pm 0.1	6.5 \pm 0.1	6.4 \pm 0.2	6.3 \pm 0.2	6.1 \pm 0.2	6.3 \pm 0.1	6.2 \pm 0.2	6.5 \pm 0.1	6.3 \pm 0.1
Evenness	0-10	0.9 \pm 0.0											
	10-20	0.9 \pm 0.0	0.8 \pm 0.1	0.9 \pm 0.0									
(d) Fungi	Abundance	6 \pm 4	5 \pm 1	3 \pm 1	5 \pm 2	4 \pm 2	5 \pm 1	4 \pm 2	5 \pm 1	7 \pm 2	8 \pm 3	4 \pm 1	6 \pm 3
	Richness	319 \pm 22	295 \pm 18	326 \pm 23	338 \pm 36	354 \pm 8	357 \pm 29	322 \pm 23	294 \pm 24	328 \pm 15	343 \pm 40	380 \pm 26	369 \pm 12
Shannon	0-10	3.6 \pm 0.3	2.9 \pm 0.5	3.2 \pm 0.7	3.3 \pm 0.2	3.3 \pm 0.5	3.4 \pm 0.4	3.5 \pm 0.3	2.9 \pm 0.2	2.8 \pm 0.0	3.5 \pm 0.1	3.5 \pm 0.3	3.6 \pm 0.3
	10-20	3.5 \pm 0.3	2.9 \pm 0.2	3.6 \pm 0.1	3.4 \pm 0.1	3.4 \pm 0.5	3.4 \pm 0.5	3.5 \pm 0.2	3.0 \pm 0.1	3.7 \pm 0.2	3.4 \pm 0.1	3.5 \pm 0.3	3.4 \pm 0.7
Evenness	0-10	0.6 \pm 0.1	0.5 \pm 0.1	0.5 \pm 0.1	0.6 \pm 0.0	0.6 \pm 0.1	0.6 \pm 0.1	0.6 \pm 0.1	0.5 \pm 0.0	0.5 \pm 0.0	0.6 \pm 0.0	0.6 \pm 0.1	0.6 \pm 0.0
	10-20	0.6 \pm 0.1	0.5 \pm 0.0	0.6 \pm 0.0	0.6 \pm 0.0	0.6 \pm 0.1	0.6 \pm 0.1	0.6 \pm 0.0	0.5 \pm 0.0	0.6 \pm 0.0	0.6 \pm 0.0	0.6 \pm 0.1	0.6 \pm 0.1

Conv: conventional agriculture, CA: conservation agriculture, OA: organic agriculture, Conv-CA: transition from conventional to conservation, Conv-OA: transition from conventional to organic, CA-OA: transition from conservation to organic.

Table 3. 4 Effect of practice intensity on (a) molecular microbial biomass, (b) ratio fungi/bacteria, (c) bacterial and (d) fungal abundance and diversity at 0-10 and 10-20 cm depth in 2020 and 2021. *t*-value and *P*-value were obtained using GLM with a quasi-Poisson error distribution. Bold values indicate significant effects ($P < 0.05$ *, < 0.01 **, < 0.001 ***). Only primary indicators having a significant ($P < 0.05$) positive (+) or negative (-) effect are reported.

	Depth	2020						2021						Indicators 2020	Indicators 2021	
		Itill		Itreat		Ifertil		Itill		Itreat		Ifertil				
		<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>			
(a) Biomass	0-10	-0.36	0.724	0.17	0.870	0.58	0.570	-0.77	0.448	-0.19	0.855	0.66	0.520	n.s.	n.s.	
	10-20	1.83	0.082	-1.03	0.318	0.74	0.468	0.58	0.566	-0.65	0.523	0.66	0.518	(+) nbOrg	(+) nbOrg	
(b) Ratio F/B	0-10	-0.51	0.616	-0.57	0.577	0.79	0.439	-0.37	0.712	-0.11	0.915	-0.60	0.555	(+) qminN	(+) qminN	
	10-20	2.85	0.010*	-2.48	0.023*	1.61	0.123	2.99	0.008**	-0.93	0.364	-1.34	0.195	(+) nbDTill, nbOrg; (-) soilP, nbFung, nbInsect	(+) nbDTill; (-) nbInsect	
(c) Bacteria	Abundance	0-10	0.14	0.888	-0.32	0.754	0.23	0.818	-1.33	0.198	-0.68	0.505	-0.42	0.678	n.s.	n.s.
	10-20	1.52	0.145	-0.64	0.530	-0.68	0.507	0.85	0.407	-0.76	0.459	0.76	0.459	n.s.	n.s.	
Richness	0-10	1.11	0.280	-0.68	0.505	-0.73	0.477	1.03	0.316	-0.08	0.935	1.03	0.316	(-) qminN	n.s.	
	10-20	1.84	0.081	-0.83	0.416	-1.00	0.329	1.28	0.216	-0.06	0.955	0.35	0.730	(+) nbDTill; (-) qminN	n.s.	
Shannon	0-10	1.30	0.209	-0.62	0.546	0.63	0.533	1.23	0.233	-1.13	0.273	1.24	0.230	(+) resExp; (-) qminN	(+) resExp	
	10-20	2.45	0.024*	-0.81	0.425	0.04	0.970	1.94	0.068	-0.41	0.684	0.77	0.454	n.s.	n.s.	
Evenness	0-10	1.05	0.309	-0.40	0.696	1.52	0.146	0.92	0.398	-1.34	0.197	0.94	0.359	(+) resExp; (-) soilP	n.s.	
	10-20	2.54	0.020*	-0.76	0.459	0.40	0.691	2.14	0.046*	-0.61	0.547	0.92	0.368	(-) soilP	(+) nbDTill, resExp	
(d) Fungi	Abundance	0-10	-0.02	0.985	-0.46	0.650	0.53	0.560	-0.93	0.363	-0.46	0.650	-0.70	0.490	n.s.	n.s.
	10-20	3.44	0.003**	-2.67	0.015*	0.89	0.387	2.33	0.031*	-0.88	0.391	0.00	0.998	(+) nbDTill, nbOrg; (-) soilP, nbFung, nbInsect	(+) nbDTill; (-) soilP	
Richness	0-10	1.90	0.072	-2.12	0.047*	0.21	0.836	2.66	0.015*	-2.58	0.018*	0.92	0.368	(-) nbHerb	(+) nbSTill, nbOrg; (-) soilP, nbHerb, nbFung, qminN	
	10-20	3.03	0.007**	-2.83	0.011*	0.06	0.955	2.54	0.020*	-1.73	0.099	0.30	0.768	(+) nbDTill; (-) soilP, nbHerb, qminN	(+) nbSTill; (-) nbFung	
Shannon	0-10	1.63	0.120	-0.29	0.775	0.93	0.362	6.05	<0.001***	-2.41	0.026*	-0.25	0.807	n.s.	(+) nbDTill, nbSTill; (-) soilP, nbFung, nbInsect	
	10-20	1.07	0.300	0.22	0.827	0.80	0.436	1.61	0.124	0.16	0.877	1.58	0.130	n.s.	n.s.	
Evenness	0-10	1.43	0.168	-0.10	0.925	0.93	0.365	5.34	<0.001***	-2.09	0.050	-0.43	0.676	n.s.	(+) nbDTill, nbSTill; (-) soilP, nbFung, nbInsect	
	10-20	0.65	0.523	0.68	0.502	0.86	0.399	1.21	0.239	0.43	0.669	1.56	0.135	(+) nbInsect	n.s.	

nbCrop: nb of crops (5 y), nbDTill: nb of deep tillage, nbSTill: nb of surface tillage, soilP: type of tillage (5 y), Itill: tillage intensity index, nbHerb: nb of herbicides, nbFung: nb of fungicides, nbInsect: nb of insecticides, Itreat: pesticide treatment intensity index, nbOrg: nb of organic matter inputs (5 y), qminN: quantity of mineral nitrogen (kg N.ha $^{-1}$), resExp: nb of residues exportations (5 y), Ifertil: fertilization intensity index, n.s.: no significant effect.

3.3.4 Bacterial and fungal diversity and community composition

In total, 7 883 189 sequences of 16S-rRNA and 6 727 895 sequences of ITS2 were retrieved from all the soil samples of the 21 plots. Archaea represented on average 48% of the 16S sequences (mostly *Nitrososphaeria* from the *Crenarchaeota* phylum), which is higher than what is reported in the literature (Karimi et al., 2018), but could be explained by the use of different versions of the SILVA database. Only 5% of ITS sequences were not assigned to the fungi kingdom. Results are presented for cropping systems (Table 3.3) and intensity indexes (Table 3.4). Additional effects observed with primary indicators of practice intensity are reported in Table 3.4.

3.3.4.1 Bacterial diversity

A total of 1668 bacterial OTU was identified and affiliated to 23 phyla, 59 classes, 103 orders, 136 families, 193 genera and 29 species of bacteria (excluding “multi-affiliations” and “unknown” taxa). Dominant bacterial phyla were *Proteobacteria* (20%-35% of relative abundance), *Bacteroidota* (5%-35%), *Acidobacteriota* (9%-24%) and *Actinobacteriota* (9%-20%) (Figure 3.1a). Other abundant phyla were *Firmicutes* (2%-26%) and *Chloroflexi* (3%-9%). The relative abundance of the main bacterial phyla was not markedly different between the different cropping systems, years or depths (Figure 3.1a).

Bacterial OTU diversity differed between cropping systems. At 0-10 cm depth, bacterial OTU richness was significantly lower in CA than in Conv-CA in both years and also than in Conv-OA in 2021 (Table 3.3c). In addition, the Shannon index of bacterial diversity was lower in CA than in Conv-OA in 2020. At 10-20 cm depth, Shannon and evenness indexes were lower in CA than in all other systems in 2020, and the Shannon index was lower in CA than in Conv-OA in 2021 (Table 3.3c). Furthermore, Shannon and evenness indexes increased under higher tillage intensity in both years at 10-20 cm depth (Table 3.4c). No other effect of practice intensity indexes was observed on bacterial diversity.

The PCoA performed on bacterial OTU demonstrated a low dissimilarity between bacterial communities in different cropping systems at 0-10 cm depth (Figure 3.2a). At 10-20 cm depth, bacterial communities in Conv-CA plots differ from the other systems (Figure 3.2c).

The redundancy analysis (RDA), conducted to assess the relation between soil properties and bacterial community composition in the studied plots, demonstrated a significant effect of many soil properties on bacterial phyla at 0-10 and 10-20 cm depth (Figure 3.3a and c). First and second axes accounted for 32% and 33% of the variance for 0-10 and 10-20 cm depth analyses respectively. The main influencing variables were soil pH, clay, coarse silt and Mn content. At 0-10 cm depth, *Proteobacteria* were related to Mn and Al contents, *Entotheonellaeota*, *Myxococcota*,

Planctomycetota and *Acidobacteriota* to clay content and pH, and *Firmicutes* to coarse silt content (Figure 3.3a). At 10-20 cm depth, *Entotheonellaeota* were related to pH, OM and clay content (Figure 3.3c).

Heatmaps of the main bacterial classes at 0-10 and 10-20 cm in 2020 and in 2021 (Figure 3.4) showed that several taxa had a higher abundance in Conv plots (*Acidimicrobiia*, *Vicinamibacteria*, *MB-A2-108* and *bacteriap25*). At 10-20 cm, *KDA-96*, *Methylomirabilia* and *Nitrospira* presented a high abundance in all Conv-CA plots (Figure 3.4, Figure S3.2). In addition, tillage intensity had an influence on several bacterial classes at 10-20 cm depth (Figure 3.4, Figure S3.3). The relative abundances of *Actinobacteria*, *Cyanobacteriia* and *Polyangia* tend to be higher under high tillage intensity. Inversely, *KD4-96*, *Methylomirabilia*, *Gitt-GS-136* and *Nitrospira* were encountered under lower tillage intensity. One CA plot (CA2) had a very high abundance of *Acidobacteriae* and *Ktedonobacteria* in relation to the low pH of soil (Figure 3.4).

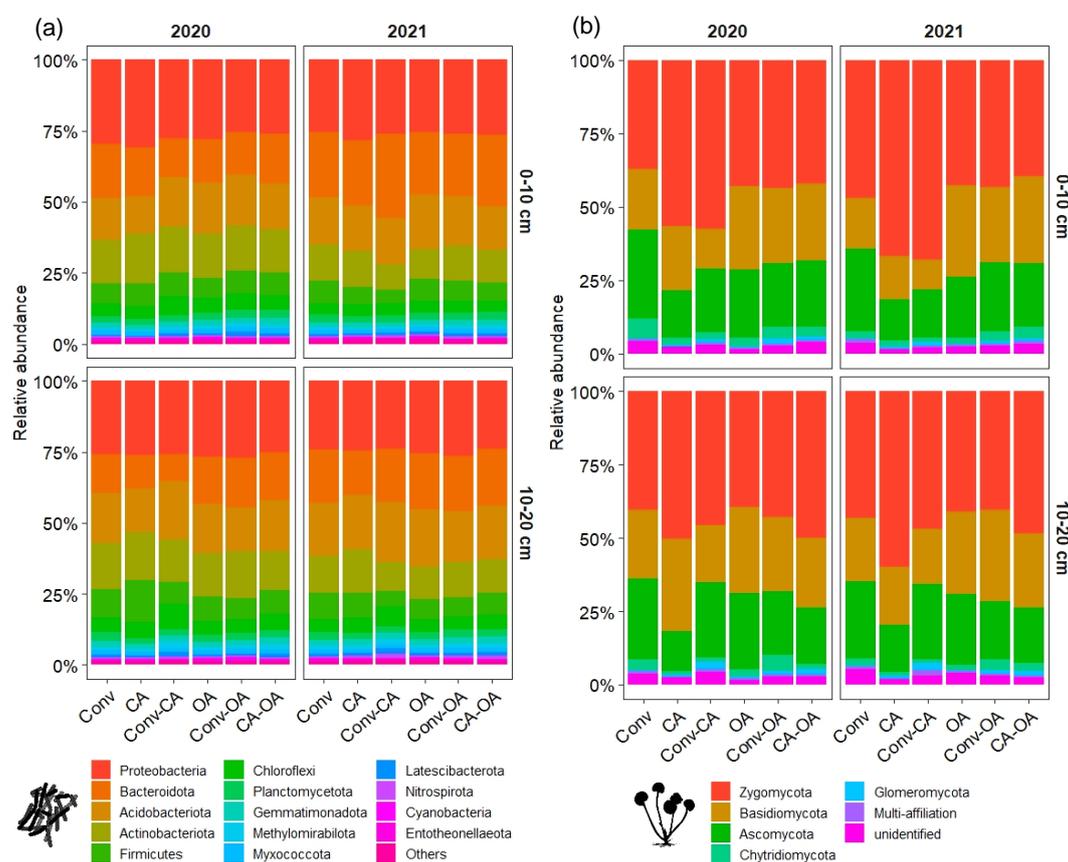


Figure 3. 1 Relative abundance of (a) bacterial and (b) fungal phyla at 0-10 and 10-20 cm depth in 2020 and 2021. Taxa with a relative abundance of less than 1% in all samples are grouped as “Others”. Fungal OTU that could not be analyzed or identified are grouped as “Unidentified” or “Multi-affiliation”. Conv, CA and OA: conventional, conservation and organic agriculture; Conv-CA, Conv-OA and CA-OA: initial-recent system transitions.

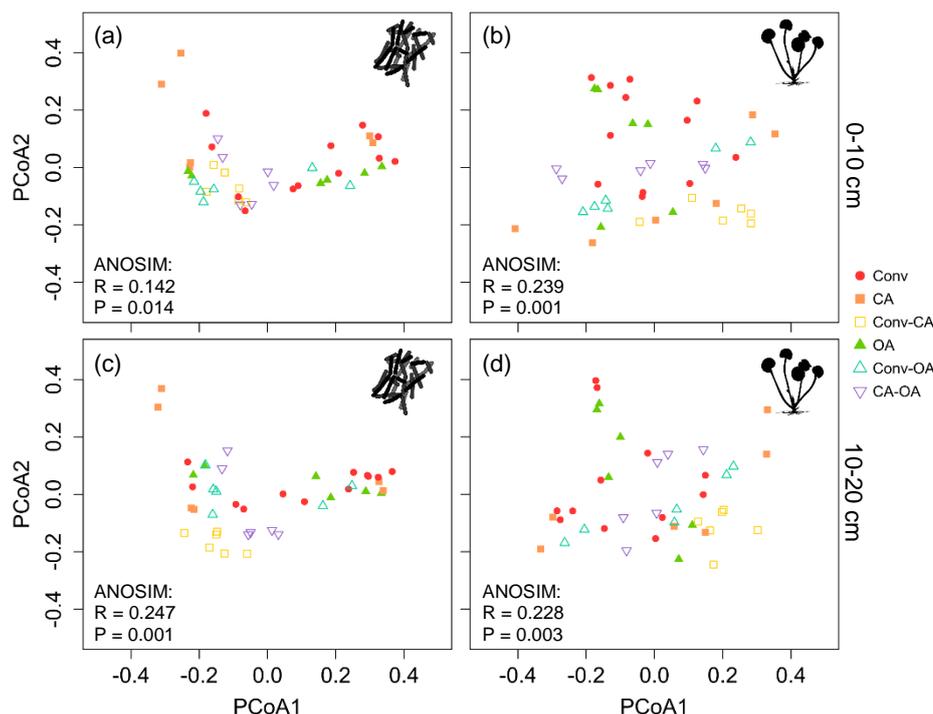


Figure 3.2 Principal coordinate analysis (PCoA) based on Bray-Curtis distance of bacterial and fungal OTU at (a) and (b) 0-10 cm depth, (c) and (d) 10-20 cm depth. Each point represents the average value for a plot in either 2020 or 2021. Colors and symbols represents the different cropping systems as reported in the legend. Conv, CA and OA: conventional, conservation and organic agriculture; Conv-CA, Conv-OA and CA-OA: initial-recent system transitions.

3.3.4.2 Fungal diversity

A total of 789 fungal OTU were obtained and affiliated to 5 phyla, 24 classes, 60 orders, 102 families, 140 genera and 285 species of fungi (excluding “multi-affiliations” and “unknown” taxa). Dominant fungi phyla were *Zygomycota* (20%-74%), *Basidiomycota* (6%-49%) and *Ascomycota* (8%-39%) (Figure 3.1b). The relative abundance of *Zygomycota* was on average higher in CA and Conv-CA than in other systems at 0-10 cm depth (Figure 3.1b). *Zygomycota* were also more abundant in CA than in other systems at 10-20 cm depth, with a similar but slight difference for Conv-CA and CA-OA. In addition, *Basidiomycota* relative abundance was the lowest in Conv-CA at 0-10 cm depth, and *Ascomycota* were slightly more represented under Conv at 0-10 cm depth (Figure 3.1b).

At 0-10 cm depth, fungal OTU richness was lower in CA than in Conv-OA and CA-OA in both years and was also lower in Conv than in Conv-OA in 2021 (Table 3.3d). Shannon and evenness indexes of fungal diversity were significantly lower in CA and Conv-CA in 2021. At 10-20 cm depth, fungal OTU richness was lower in CA than in Conv, OA, Conv-OA and CA-OA and lower in Conv and Conv-CA than in Conv-OA in 2020, whereas fungal richness was lower in CA than in OA and CA-OA in 2021 (Table 3.3d). Furthermore, fungal OTU richness increased under higher tillage intensity and lower treatment intensity for all depths and years (Table 3.4d). Shannon and

evenness indexes followed a similar response to tillage and treatments but at 0-10 cm depth and in 2021 only (Table 3.4d).

The PCoA demonstrated a dissimilarity between fungal communities of different cropping systems, separating communities observed in Conv-CA with the ones observed in Conv, Conv-OA and CA-OA at 0-10 and 10-20 cm depth (Figure 3.2b and d).

The RDA demonstrated that a limited number of soil properties influenced fungal phyla at 0-10 and 10-20 cm depth (Figure 3.3b and d). RDA first and second axes explained 31% and 30% of variance for 0-10 and 10-20 cm depth analyses respectively. Fungal phyla composition was affected by P, Mg, fine silt and coarse sand content, and not significantly by organic matter content (Figure 3.3b). The P content of soil mostly influenced *Basidiomycota* (Figure 3.3b).

The heatmaps of the main fungal classes at 0-10 and 10-20 cm in 2020 and in 2021 (Figure 3.4) showed few effects of cropping systems (Figure S3.4) or tillage intensity (Figure S3.5) on the relative abundance of fungal classes. A slightly higher abundance of *Sordariomycetes* was observed in Conv plots than in other plots at 0-10 cm depth (Figure 3.4).

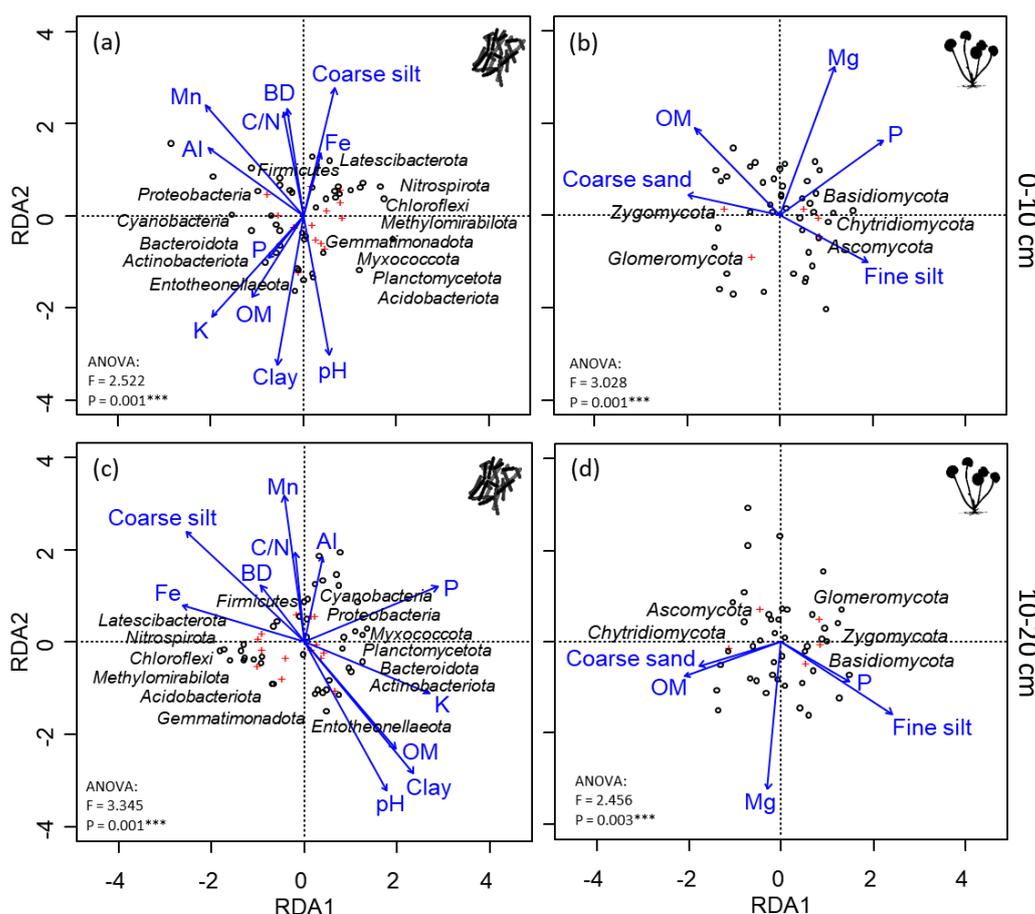


Figure 3. 3 Redundancy analysis (RDA) showing the correlations between bacterial and fungal phyla and soil parameters at (a) and (b) 0-10 cm depth, (c) and (d) 10-20 cm depth. Points represent plots in either 2020 or 2021. Red crosses are phyla. Al: aluminium, BD: bulk density, C:N: ratio carbon/nitrogen, Fe: iron, K: potassium, Mg: magnesium, Mn: manganese, OM: organic matter, P: phosphorus.

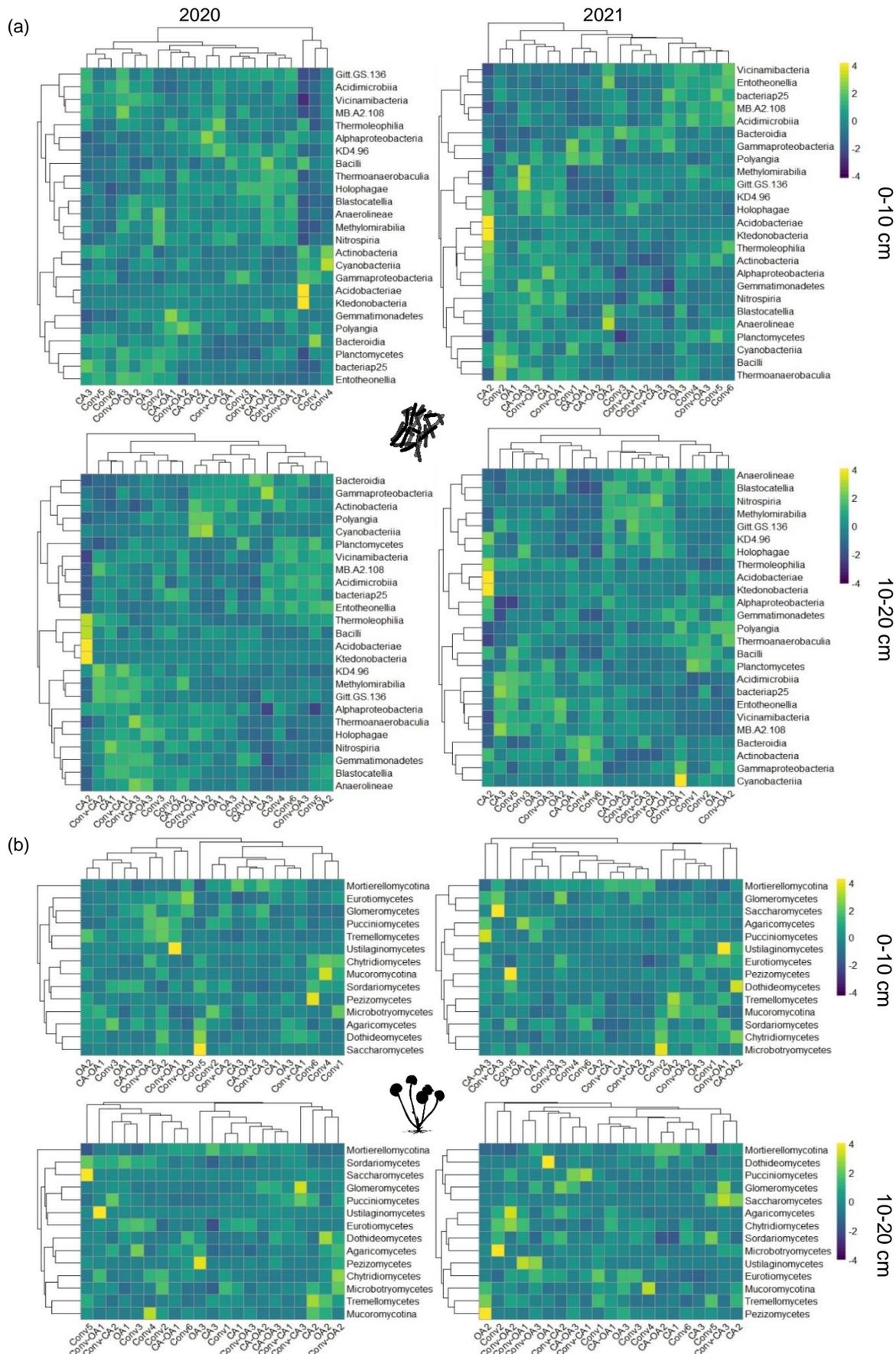


Figure 3. 4 Hierarchically clustered heatmaps of the relative abundance of the main (a) bacterial and (b) fungal classes. Only classes representing more than 1% of total abundance in at least one sample are presented. Columns correspond to plots named according to the cropping system they represent.

3.3.5 Bacterial functional traits

The BactoTraits database provided trait profiles for 81% of the bacterial OTU obtained from our soil samples. Regarding trait attributes, values were missing for 321 to 1245 OTU.

The PCA on bacterial traits showed that functional communities of bacteria in Conv-CA differed from communities in Conv and CA at 10-20 cm depth in both years (Figure 3.5c and d). In addition, all transitioning systems (Conv-CA, Conv-OA and CA-OA) had functional communities that differed from the ones observed in Conv at 0-10 cm depth in but in 2020 only (Figure 3.5a).

The traits influenced by cropping systems irrespective of year and depth were delta and range of growth temperature, pigmentation, %GC in genome and trophic type (Table S3.1). Trait attributes differed between cropping systems depending on depth and year (Table S3.1), but some patterns were observed in both years with significant differences mainly observed between Conv and Conv-CA (Figure 3.6). At 0-10 cm, Conv-CA had less organo-trophic bacteria than Conv (Figure 3.6a). At 10-20 cm, Conv-CA systems had more bacteria with a GC content of 57-66 % and a photo-trophic type compared to Conv, and less bacteria with high growth temperatures (30-34°C, 27-30°C optimum) and an organo-trophic regime (Figure 3.6b). Besides, Conv-CA benefited to bacteria with a filamentous shape and a green pigmentation more than the other systems (Figure 3.6b). In addition, more bacteria with a range of high growth temperature (30-34°C) were encountered in OA than in Conv-CA, and in Conv than in CA (Figure 3.6b).

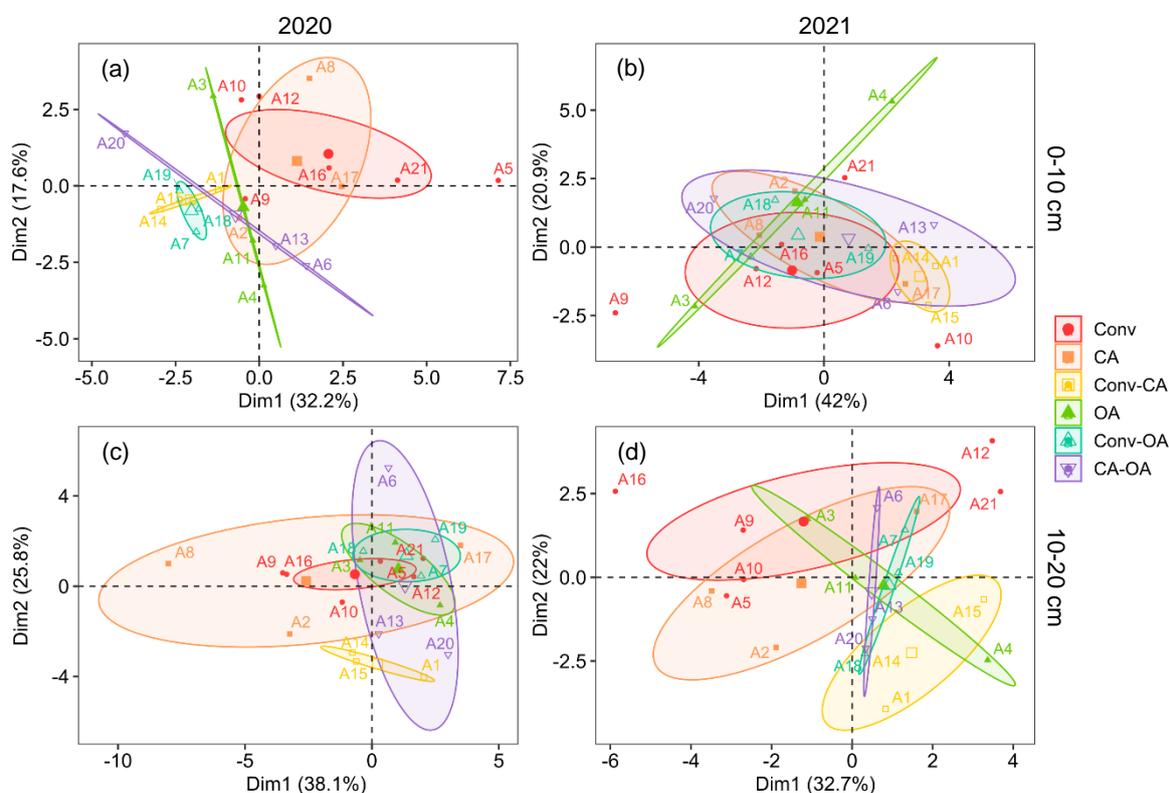


Figure 3.5 PCA on bacterial traits, for (a) and (b) at 0-10 cm and for (c) and (d) at 0-20 cm depth in 2020 and in 2021. Ellipses represent cropping systems. Small points are plots and large points are ellipse centroids. Conv, CA and OA: conventional, conservation and organic agriculture; Conv-CA, Conv-OA and CA-OA: initial-recent system transitions.

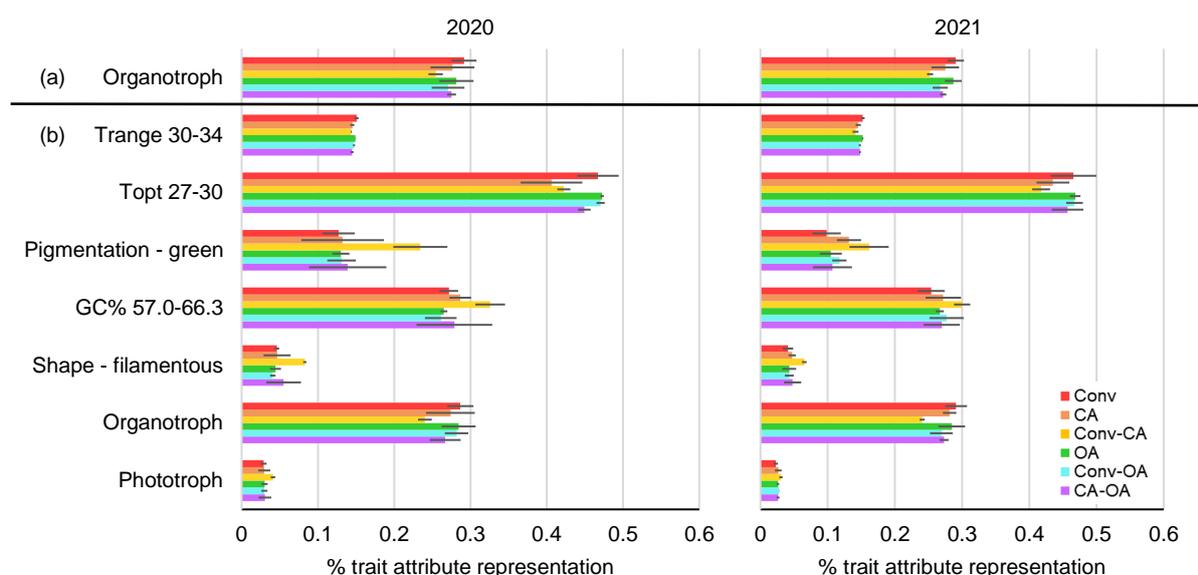


Figure 3. 6 Bacterial traits influenced by cropping systems at (a) 0-10 cm and (b) 10-20 cm depth in 2020 and in 2021. Only trait attributes significantly different between cropping systems both in 2020 and in 2021 are represented. All trait attributes influenced by cropping systems are reported in Table S3.1.

Among practices, tillage intensity influenced the highest number of traits with consistent effects in 2020 and 2021 (Table 3.5). At 0-10 cm, we observed less bacteria with a delta NaCl of 1-3% under higher tillage intensity (Table 3.5a). At 10-20 cm, an increase in tillage intensity was related to the presence of bacteria with a delta pH of 2-3, delta temperature of 20-30°C, temperature range of 30-34°C and optimum of 27-30°C, yellow pigmentation, rod shape and methyl-trophic type (Table 3.5b). Conversely, high tillage intensity was also associated with a decrease in bacteria with a NaCl optimum above 8%, delta temperature of 1-5°C and filamentous shape (Table 3.5b). An increase in treatment intensity promoted non-motile bacteria at 0-10 cm depth (Table 3.5a). Fertilization intensity has poor and no consistent effects across the years.

Table 3. 5 Effect of tillage and pesticide treatment intensity on bacterial traits at (a) 0-10 cm and (b) 10-20 cm depth in 2020 and 2021. *t*-value and *P*-value were obtained using GLM with a quasi-Poisson error distribution. Only trait attributes with a significant effect both in 2020 and in 2021 are presented ($P < 0.05$ *, < 0.01 **, < 0.001 ***).

	2020				2021			
	Itill		Itreat		Itill		Itreat	
	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>
(a) 0-10 cm								
ΔNaCl 1-3	-2.55	0.020*			-2.80	0.011*		
Non-motile			-2.17	0.042*			-2.28	0.034*
(b) 10-20 cm								
ΔpH 2-3	2.41	0.026*			2.17	0.043*		
NaCl _{opt} >8	-2.48	0.023*			-2.39	0.027*		
ΔT 20-30	3.07	0.006**			3.01	0.007**		
ΔT 1-5	-2.88	0.010*			-3.37	0.003**		
Trange 30-34	2.25	0.037*			2.40	0.027*		
Topt 27-30	3.57	0.002**			3.39	0.003*		
Yellow pigmentation	2.58	0.018*			2.35	0.030*		
Filamentous shape	-2.11	0.049*			-2.48	0.023*		
Rod shape	2.36	0.029*			2.39	0.027*		
Methylotroph	3.26	0.004**			2.89	0.009**		

ΔpH: delta of pH for growth; NaCl_{opt}, ΔNaCl: optimal and delta of NaCl concentration for growth; Topt, Trange, ΔT: optimal, delta, range of temperature for growth.

3.4 Discussion

Our study explores the effect of cropping systems and practice intensity on soil bacterial and fungal communities at different depths and for two consecutive autumns. Although cropping systems or practice intensity showed no effect on microbial biomass and bacterial abundance, they significantly affected fungal abundance, bacterial and fungal diversity, as well as bacterial functional traits.

3.4.1 Effect of soil properties and cropping systems on bacteria and fungi communities

Soil properties in conservation systems differ from the other cropping systems, because of the accumulation of organic matter at the surface and changes in soil bulk density. In the absence of tillage, soil remains undisturbed and plant litter decomposition occurs at the soil surface (Winter et al., 1990). As soil is not mixed, soil bulk density increases with depth while the number and size of soil pores decreases (Mielke et al., 1986). In addition, no-tillage or reduced tillage are responsible for an increase in soil moisture and smaller fluctuations of soil temperature, which may benefit the activity of soil microorganisms (Kladivko, 2001). However, here, we observed that soil chemical composition varied more according to plots than to cropping systems.

In our study, the microbial biomass was relatively low compared to the mean value obtained for cropping systems ($38 \mu\text{g DNA.g}^{-1}$ soil) over many sites in France (Briat and Job, 2017). The microbial biomass is considered to be an early indicator of changes in soil quality, notably due to agricultural practices (Dequiedt et al., 2011; Schloter et al., 2018). However, as reported in other studies (Scopel et al., 2013; Dunn et al., 2021), our results do not show effect of cropping systems on molecular microbial biomass at any depth for the first 20 cm.

Bacterial abundance was similar in all cropping systems, while fungal abundance was higher in organic systems than in recent conservation systems at 10-20 cm depth in 2020. In line with this, organic farming was reported to benefit fungal abundance, mostly because of higher organic matter inputs (Hole et al., 2005; Birkhofer et al., 2008). However, our results contrast with those of Henneron et al. (2015) who reported differences in bacterial abundance, which gradually increased in conventional, organic and conservation systems, and a higher fungal abundance in conservation than in organic (without exogenous organic matter addition) and conventional systems. In addition, we found no difference in fungal abundance between conservation and conventional systems whereas fungal abundance was reported to increase during the transition to no-tillage systems (Spurgeon et al., 2013). Furthermore, the observed effects of cropping systems on fungal abundance but not on bacterial abundance could be explained by a larger impact of crop management on fungi than on bacteria. Indeed, fungi may respond quickly to

changes due to disturbances whereas bacteria are more affected by soil parameters (Coller et al., 2022).

Numerous soil properties were reported to influence soil bacterial and fungal communities (Bahram et al., 2018; Karimi et al., 2018; Delgado-Baquerizo and Eldridge, 2019). We observed that the bacterial community composition was influenced by a large set of soil properties (e.g. pH, soil texture, C/N, Al) while fungal communities responded to a limited number of properties such as soil texture and P and Mg contents. Previous studies similarly reported a major influence of soil P content on fungal communities (Chen et al., 2020; Zhang et al., 2022).

Bacterial and fungal community compositions were more variable within than between cropping systems, mostly because of their response to soil properties. This variability was exacerbated in stable conservation systems due to unexpected differences in soil properties between plots (e.g. Al and CaCO₃ contents). However, plots under recent conservation systems presented similar microbial communities and differed from all the other systems. This may indicate transitional changes in the microbial community during the few years after conversion to conservation agriculture, notably explained by a shift in soil properties related to no-tillage. Indeed, during the transition from conventional to no-tillage, a shift in the distribution of soil pores occurs (Kay and VandenBygaart, 2002), allowing for a better circulation of water and air in soil (Winter et al., 1990) and hence changes in soil microbial habitat. Overall, our systems transitioning to organic or conservation agriculture showed differences with their initial systems. However, the lack of studies on the effect of the transition to alternative systems, especially to conservation systems, prevents further interpretation of the results at the system level. Thus, more studies are required to understand the changes in microbial communities that occur during the transition from conventional to alternative systems and from one alternative to another.

Bacterial and fungal diversity were the highest in recent organic systems and the lowest under conservation systems irrespective of year and depth. Previous studies on the transition to organic farming similarly reported an increase in bacterial diversity with year since conversion (Shu et al., 2012) but a limited effect on fungal species (Jabbour and Barbercheck, 2009). Regarding conservation systems, results observed in the literature are more contrasted. Previous studies reported no-tillage and conservation systems to have no significant effect on microbial diversity (Kraut-Cohen et al., 2020) and fungal diversity (Zhang et al., 2022). However, conservation agriculture was reported to benefit arbuscular mycorrhizal fungi (Mhlanga et al., 2022) and previous reviews reported an overall benefit on microbial diversity (Palm et al., 2014; Li et al., 2020). Therefore, the effect of conservation systems on fungal diversity is complex and should be assessed at the practice level.

The most abundant bacterial phyla observed in all cropping systems were *Proteobacteria*, *Bacteroidota*, *Acidobacteriota* and *Actinobacteriota*, which is consistent with the main phyla

observed in French soils (Karimi et al., 2018). Also in agreement with previous studies, the main fungal phyla in all cropping systems were *Zygomycota*, *Basidiomycota* and *Ascomycota* (Song et al., 2018; Wang et al., 2021). Our results showed no effect of cropping systems on the relative abundance of the main bacterial phyla, as previously reported in other studies (Essel et al., 2019; Bill et al., 2021). This may be explained by a stronger effect of soil parameters than cropping systems on bacterial communities. In contrast, we observed that cropping systems influenced the relative abundance of the main fungal phyla, with a higher relative abundance of *Zygomycota* under conservation systems, mostly at 0-10 cm depth. In our study, the *Zygomycota* phylum was mostly represented by fungi of the *Mortierella* genus, which were reported to be related to surface organic matter and to slow litter degradation at the soil surface (Li et al., 2018). In addition, we observed a higher relative abundance of *Ascomycota* in conventional systems at 0-10 cm depth. It may be explained as conventional systems mostly relied on mineral fertilizers that are reported to promote a higher proportion of *Ascomycota* on the long-term (Chen et al., 2021). Furthermore, the relative abundance of bacterial and fungal classes varied between plots. However, we found no clear relation with cropping systems. Several bacterial classes had a very high relative abundance in some plots, in relation to differences in soil properties (e.g. pH in A8 plot).

3.4.2 Tillage intensity as a driver of the effect of cropping systems on bacteria and fungi communities

We observed that tillage influenced fungal abundance as well as bacterial and fungal diversity, being the main factor influencing microbial communities as shown in previous studies (Le Guillou et al., 2019). An effect of tillage on soil microbial communities was repeatedly noted (Wardle, 1995; Young and Ritz, 2000; Anderson et al., 2017; Wang et al., 2021) and both long-term tillage and single tillage operations were reported to affect soil microbial abundance (Kraut-Cohen et al., 2020). More impacts of tillage were reported for fungi than for bacteria. This is due to the physical damage caused by tillage on the mat formed by hyphal filaments, which reduces fungal growth and mobility in soil (Stubbs et al., 2004). Negative effects were reported in particular for arbuscular mycorrhizal fungi (Kabir et al., 1998; Jansa et al., 2003). Conversely, other studies found larger effects of tillage on bacteria than on fungi (Anderson et al., 2017). However, in our study, we observed beneficial effects of tillage intensity on bacterial richness at 10-20 cm depth, and on fungal abundance and richness at all depths. This may be explained as tillage mix crop residues or organic matter inputs (e.g. manure, compost) with soil, burying substrate deeper in soil. As the contact area between soil and substrate increases, soil microorganisms are able to better colonize and degrade organic matter (Stubbs et al., 2004). In addition, our results show that tillage is a major factor explaining the relative abundance of

bacterial classes in different plots, confirming its influence on bacterial community structure (Romero-Salas et al., 2021).

Pesticide treatment intensity showed negative effects on fungal abundance. However, it could be a confounding effect with tillage intensity. Indeed, treatment intensity is inversely related to tillage intensity in most cropping systems. However, negative effects of pesticide treatments on fungi were previously reported (Beare et al., 1993; Bünemann et al., 2006).

Fertilization intensity had no impact on microbial parameters, contrary to our expectations and to previous findings (Hole et al., 2005; Bill et al., 2021; Rachwał et al., 2021). This lack of effect could be related to the response time of the microbial community, suggesting that the effect of fertilization intensity may be observed at a longer term (i.e. 20 years) than investigated in our study (i.e. 5 years) (Geisseler and Scow, 2014; Schmid et al., 2018). In addition, it may be necessary to take into account the type (i.e. organic or mineral, quality) and the amount of fertilizers in order to assess fertilization effects on microbial communities. Indeed, we observed a higher microbial biomass at 10-20 cm depth with a higher number of organic matter inputs over 5 years, most likely because it provides an additional source of C, N, P and K nutrients (Bünemann et al., 2006; Lejon et al., 2007; Zhong et al., 2010).

Overall, our study demonstrated that agricultural management has a complex effect on microbial communities, requiring to take into account the diversity of practices within cropping systems, their direct impacts on microorganisms, as well as their indirect impacts occurring through changes in soil properties.

3.4.3 Bacterial functional traits: a step toward the understanding of the effect of cropping systems and practice intensity on soil microorganisms

Functional traits were suggested as a mean to improve our understanding of the mechanisms influencing soil bacterial community composition (Romillac and Santorufo, 2021). In our study, we investigated changes in functional traits within the bacterial community of different cropping systems. In addition, as suggested by Romillac and Santorufo (2021), we observed changes in trait values along an environmental gradient, here represented by tillage, pesticide treatment and fertilization intensity and the associated effects on soil. The functions and environmental conditions related to traits included in BactoTraits are described in details in Cébron et al. (2021). Here, we aim to illustrate the potential of bacterial traits to better explain changes occurring in soil bacterial communities between different cropping systems.

In our study, cropping systems influenced a wide range of bacterial traits. Traits most sensitive to cropping systems, irrespective of year and depth, were traits associated to growth temperature and pH, pigmentation of colonies, %GC in the genome, trophic type and cell shape.

Functional communities and associated traits in recent conservation systems differed from conventional systems at all depths, and from stable conservation systems at 10-20 cm depth. Differences in functional communities were associated to differences in several bacterial traits (optimum and range of growth temperature, %GC, trophic type, shape, pigmentation) irrespective of the year and depth.

In recent conservation systems, we observed a lower representation of organo-trophic bacteria than in conventional systems at 0-10 and 10-20 cm depth. This change in trophic type may be explained by a niche differentiation as residues accumulate at the soil surface and provide a new C source for bacteria (Thies and Grossman, 2006), with a progressive adaptation of the bacterial community. At 10-20 cm depth, we observed a lower representation of bacteria associated to a high growth temperature (30-34°C with 27-30°C optimum) and a high %GC (57-66) in recent conservation than in conventional and stable organic systems. However, we also observed that bacteria growing under extreme temperatures (> 40°C) tended to increase in recent conservation systems. The tolerance to high temperatures and the high GC content both indicate a high plasticity of bacteria and their ability to adapt to environmental changes (Pettersson and Bååth, 2003; Nishida, 2012). Recent conservation systems also presented a higher share of bacteria with a filamentous shape, which have the ability to increase their surface of contact with soil and may benefit from a better nutrient access and surface attachment (Young, 2006). In addition, a higher representation of green colonies and photo-trophic bacteria suggested that even at 10-20 cm depth, bacteria may present adaptation to bright environments. Overall, bacterial traits observed in recent conservation systems showed a high plasticity of bacteria regarding environmental changes, with a better potential for adaptation and access to nutrients.

Considering practice intensity as an environmental gradient in cropping systems, we found tillage intensity to have the highest impact on bacterial functional communities. Tillage influenced bacterial traits, mostly at 10-20 cm depth, such as growth pH and temperature, pigmentation, shape and trophic type. An increase in tillage intensity notably favored rod shape bacteria over filamentous bacteria, and hence better dispersal abilities at the expense of surface attachment and nutrient access (Young, 2006). In addition, tillage favored bacteria with high range (27-34°C) and extent (20-30°C) of growth temperature, indicative of a higher plasticity of bacteria (Pettersson and Bååth, 2003), which may have been selected following successive soil disturbances and a decrease in soil protection (i.e. no mulch cover). The increase in yellow bacterial colonies with tillage could be related to soil inversion that bring bacteria at the surface and under light, hence requiring protection against UV and desiccation (Wada et al., 2013). Tillage also favors methyl-trophic bacteria and we suggest that this indicates a joint increase in methanogens microorganisms in tilled soils, in agreement with previous studies reporting tilled soils to emit more methane than soils under no-tillage (Ussiri et al., 2009; Abdalla et al., 2013). Furthermore,

pesticide treatment intensity affected bacterial functional community but mostly regarding bacterial motility. An increase in pesticide treatment intensity favored motile over non-motile bacteria. Motile bacteria were reported to respond rapidly to changes in physico-chemical parameters by moving toward a better environment (Alexandre et al., 2004). Overall, soils subjected to high tillage and pesticide treatment intensity favored r-strategists bacteria associated to the “stress-tolerant” and “colonizers” functional groups (Cébron et al., 2021).

Our results demonstrated that bacterial functional traits provide important insight on the effect of crop management on bacterial communities. Indeed, traits responded to cropping systems and practice intensity more than bacterial taxonomic groups or total abundance. In addition, bacterial functional traits appear relevant for studies assessing the changes occurring in bacterial communities during the transition to alternative systems, or in relation to tillage intensity. Furthermore, although part of the bacterial traits responded to both cropping systems and practices, several traits were only influenced by a cropping system or by a given practice. This again emphasizes on the need to assess the diversity of practices within cropping systems in order to have a full overview of their effect on the soil microbial community. Finally, it would also be relevant to develop similar approaches for fungi.

3.5 Conclusion

Soil properties, as well as cropping systems and tillage intensity are major drivers of bacterial and fungal communities in agricultural soils. Furthermore, the transition toward organic systems has the potential to increase fungal abundance and microbial diversity compared to conventional and conservation systems. These differences are mostly explained by a positive effect of tillage on microbial communities at 10-20 cm depth. In addition, bacterial functional traits appear relevant to further our understanding of the mechanisms driving soil microbial community and soil functioning in cropping systems. Indeed, we observed major differences in bacterial functional communities in recent conservation systems compared to other systems. Therefore, we advocate for the need of studies implementing the functional trait approach to characterize the effect of alternative cropping systems on the microbial community. The variability of microbial communities remains high within systems, thus future studies should investigate the response of microbial communities and bacterial traits for different years, seasons and stages of the crop cycle.

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Transition toward the chapter 4

In the chapters 1 to 3, we assessed the effects of cropping systems and practice intensity on the density, diversity and community structure of mesofauna, macrofauna and microorganisms based on the samplings performed in 2020 and 2021. We showed that cropping systems influence soil organisms but that the response vary according to year and taxa (Figure T.1). In addition, we observed that practice intensity indicators allow better characterizing the effects of cropping systems on soil organisms. In the chapter 4, we provide a synthesis of the effect of cropping systems on the density of the three size groups of soil organisms and additionally assess the effect of practice intensity on the interactions between the main trophic groups of the soil food web.

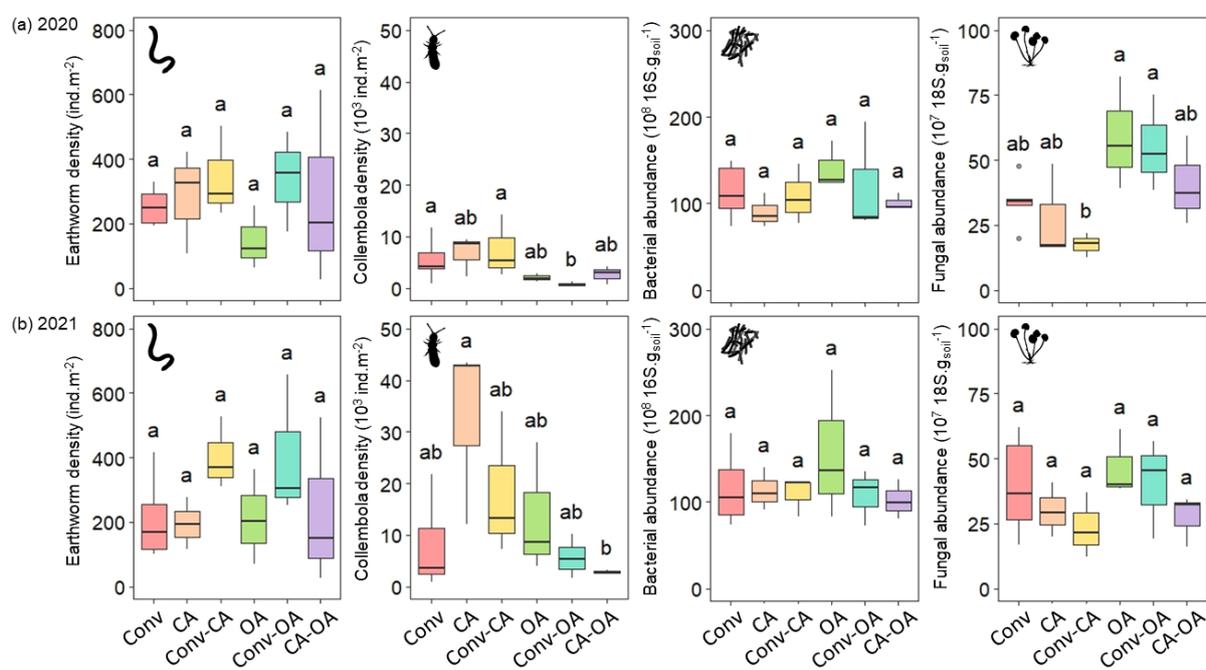


Figure T. 1 Density of earthworms, Collembola, bacteria and fungi in cropping systems in 2020 and 2021 as reported in the chapters 1 to 3. Bacterial and fungal abundance are presented at 10-20 cm depth. Conv, CA and OA: conventional, conservation and organic agriculture; Conv-CA, Conv-OA and CA-OA: initial-recent system transitions.

———— Chapter 4 ————

Toward an assessment of multiple soil taxa and their interactions in alternative and transitioning cropping systems

Juliette Chassain¹, Sophie Joimel¹, Tania de Almeida², Laure Vieublé Gonod¹

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¹ Université Paris-Saclay, INRAE, AgroParisTech, UMR ECOSYS, 91120, Palaiseau, France

² Université de Lille, Laboratoire Génie Civil & Géo-Environnement, 59655, Villeneuve d'Ascq Cedex, France

Abstract

Intensive agriculture efficiently increased crop yield in the last century but at the expense of soil biodiversity. The development of alternative systems relying on low chemical and physical disturbances such as organic and conservation agriculture could help to slow down biodiversity erosion. However, few studies were conducted on the effects of alternative systems on multiple groups of soil organisms and on their interactions, nor on the changes occurring during the transition toward these alternatives. In this study, we compared the effects of different alternative cropping systems, stable or in transition, on soil microorganisms, mesofauna and macrofauna. In addition, we investigated the effect of practice intensity on the interactions between soil organisms within the soil food web. Soil organisms were sampled in 21 plots under real conditions in autumn 2020 and 2021. Cropping systems were under conventional, organic or conservation agriculture and either stable (≥ 7 years) or in transition (≤ 3 years). Effect sizes were calculated to compare the effects of alternative and conventional systems, as well as the transitioning and initial systems, on soil microorganisms, mesofauna and macrofauna. Structural equation modeling (SEM) was conducted to assess the effect of tillage, pesticide treatment and organic fertilization intensity on soil organic matter and trophic groups of soil organisms.

Our results show a positive effect of stable conservation systems on the density of Collembola, Coleoptera larvae and Gastropoda compared to conventional agriculture. Similarly, systems in transition toward conservation agriculture had a positive effect on the density of Coleoptera larvae and earthworms, and a negative effect on fungal abundance at 10-20 cm depth. Stable organic systems showed no significant difference with conventional systems but tended to promote soil microorganisms, whereas transitioning organic systems had a positive effect on earthworm density. Conservation and organic agriculture had an overall mean positive effect on the density of soil organisms, and conservation systems tended to have the largest benefits. Tillage intensity was the main driver of the negative effect of cropping systems on soil organisms with cascading effects through the soil food web. Organic matter inputs promoted the density of bacteria and macrofauna detritivores. In addition, mesofauna detritivores were observed to have a central place in the soil food web of agroecosystems. Overall, our study demonstrates that conservation systems and low tillage intensity promote the density of several groups of soil organisms and their interactions within the soil food web with additional effects of other practices and soil properties.

4.1 Introduction

Soil organisms interact at different spatio-temporal scales and are involved in many soil functions, thus driving ecosystem services in agricultural areas (Kibblewhite et al., 2008). However, intensive agriculture is considered as a main threat to soil biodiversity (Gardi et al., 2013). Indeed, intensive systems are responsible for a low density and diversity of soil organisms due to a large range of soil disturbances (Ponge et al., 2013; Tsiafouli et al., 2015). The development of alternative systems relying on lower chemical and physical disturbances, such as organic and conservation systems, aims to reduce the pressures exerted by agriculture on soils and could benefit its biodiversity.

In the last decades, an increasing number of studies have been conducted on the effects of alternative systems on the density and diversity on soil organisms (Christel et al., 2021). In organic systems, soil organisms benefit from the absence of pesticides and from organic matter inputs (Bettiol et al., 2002; Maeder et al., 2002; Birkhofer et al., 2008a). On the other hand, conservation systems may benefit soil organisms by the use of no-tillage, permanent soil cover and longer crop rotations (Kladivko, 2001; Coulibaly et al., 2017; Dulaurent et al., 2022). Yet, studies comparing the effects of cropping systems on multiple taxa are scarce. For instance, Henneron et al. (2015) assessed the effects conventional, organic and conservation systems on microorganisms, microfauna and macrofauna. They found benefits of both organic and conservation systems on the density of soil organisms with an overall higher enhancement in conservation than in organic systems. These results were however observed on an experimental site where organic systems do not rely on organic matter inputs. In addition, changes occurring during the transition toward alternative systems remain poorly understood (Pelosi et al., 2015), with in particular a lack of studies on the transition toward conservation agriculture.

The nature and the intensity of the effects of cropping systems and individual practices vary with soil organisms depending in particular on their size and trophic level (Kladivko, 2001; Coudrain et al., 2016; Coller et al., 2022). Disturbances caused by agricultural practices can affect the density or diversity of one trophic group and this change may cascade across trophic levels in the soil food web (Kulmatiski et al., 2014; Tsiafouli et al., 2015). Indeed, soil biodiversity is influenced by trophic interactions and by the active and passive dispersion and modification of the soil habitat by large organisms (Wardle, 2006). For instance, soil detritivores are feeding on microorganisms and regulate their density, diversity and activity (Seastedt, 1984; Kaneda and Kaneko, 2008; Culliney, 2013). They in turn serve as prey for generalist predators (Birkhofer et al., 2008b). In addition, soil invertebrates alter the physical environment through the formation of structures (e.g. fecal pellets, casts, burrows) serving as habitat or promoting the displacement of other organisms (Lavelle and Spain, 2001; Wardle, 2006). Therefore, it is necessary to

understand the effects of agricultural practices on multiple taxa of soil organisms and on the soil food web in order to promote systems relying on strong soil biodiversity. Recent studies showed a simplification of the soil food web under intensive agriculture compared to other land-uses (Tsiafouli et al., 2015; Creamer et al., 2016). Yet, few studies assessed the effects of different cropping systems on soil organisms by considering their interactions within the soil food web.

The assessment of interactions between soil organisms is complex and often performed by looking at co-occurrences between organisms through correlations analyses. This approach was previously used for soil microorganisms (Faust and Raes, 2012) and in a lesser extent for soil fauna (Bell et al., 2010; Creamer et al., 2016). Another approach to assess relations between different soil components is to use structural equation modeling (SEM). SEM is currently underused in studies on soil organisms although it was proposed as a promising tool for soil ecology (Eisenhauer et al., 2015). Previous studies used SEM in particular to assess the relations between soil organisms, soil properties and crop yields (Jernigan et al., 2020; Li et al., 2021), soil nematods and soil respiration (Sun et al., 2022), grassland vegetation and soil invertebrates (Birkhofer et al., 2011) or vegetation, soil properties and soil invertebrates (Eisenhauer et al., 2013; De Almeida et al., 2020). They were also used to assess relations between agricultural practices and biotic factors (Schipanski et al., 2014; Li et al., 2021; Lu et al., 2022).

In this context, the objectives of this study were 1) to compare the effects of conventional, organic and conservation systems, stable or in transition, on multiple soil organisms (microorganisms, mesofauna, macrofauna), and 2) to assess the influence of practice intensity (tillage, pesticide treatments, organic matter inputs) on the interactions between trophic groups of soil organisms using SEM. We hypothesized that alternative cropping systems (organic or conservation) increased the density of various soil organisms compared to conventional agriculture, with higher benefits for conservation than for organic agriculture as discussed above. Furthermore, systems were expected to present different patterns of density within the soil community depending they were stable (≥ 7 years) or in transition (≤ 3 years), thus highlighting that positive or negative changes in the density of taxa observed in the first years of transition might disappear with time. Lastly, we expected the practice intensity to affect soil organisms depending on their trophic level, with potential cascading effects across the soil food web.

4.2 Materials and methods

4.2.1 Study sites

This study was conducted on 21 plots from the Paris basin (France). In this area, the climate is temperate with mean annual temperatures of 11°C and 600-700 mm of mean annual

precipitations. All plots were cropped with winter wheat during the 2020-2021 crop cycle and with various crops or cover crops in 2021-2022. Soils were silty to clay-silty, with a mean pH of 5.5 to 8.1 and a bulk density varying between 1.2 and 1.5 g.cm⁻³ at the 0-20 cm depth (Table 4.1).

Six cropping systems were investigated, namely stable systems (≥ 7 years) under conventional (Conv, n = 6 plots), organic (OA, n = 3) or conservation agriculture (CA, n = 3), or systems in transition (≤ 3 years) from conventional to organic (Conv-OA, n = 3), conventional to conservation (Conv-CA, n = 3) or conservation to organic agriculture (CA-OA, n = 3) (Table 4.1).

Indicators of practice intensity were computed following Büchi et al. (2019) (cf. General introduction). Composite indexes of tillage and pesticide treatment intensity were selected in order to represent the main physical and chemical soil disturbances. Here, we did not use a composite index of fertilization intensity as it previously showed few effects on soil organisms and soil properties. Instead, we considered the number of organic matter inputs over five years (nbOrg). Organic matter inputs influence soil properties and several groups of soil organisms for which they represent an additional source of directly assimilable food (cf. Chapters 1 to 3).

Table 4. 1 Characteristics of the 21 plots of the study. Soil properties are average values at 0-20 cm depth. Age corresponds to the number of years since conversion to the current system in 2020. Indicators are tillage intensity (Itill), pesticide treatment intensity (Itreat) and number of organic matter inputs over five years (nbOrg).

Plot	Clay g.kg ⁻¹	Silt g.kg ⁻¹	Sand g.kg ⁻¹	pH	SOM g.kg ⁻¹	BD g.cm ⁻³	Crop 2020	Crop 2021	System	Age	Itill 2020	Itill 2021	Itreat 2020	Itreat 2021	nbOrg 2020	nbOrg 2021
A1	155	475	54	6.9	17	1.3	wheat	wheat	Conv-CA	3	0.15	0.14	0.75	1.00	0	0
A2	210	383	119	6.5	26	1.4	wheat	cover	CA	17	0.00	0.00	0.67	0.75	1	1
A3	157	414	194	6.5	18	1.5	wheat	wheat	OA	20	0.44	0.65	0.00	0.00	3	3
A4	433	240	91	7.9	34	1.2	wheat	bare	OA	19	0.92	0.66	0.00	0.00	6	6
A5	210	448	52	6.5	22	1.4	wheat	barley	Conv	20	0.34	0.30	0.50	0.81	1	1
A6	279	377	32	6.6	21	1.3	wheat	bare	CA-OA	2	0.68	0.52	0.00	0.00	3	4
A7	174	454	63	6.7	23	1.3	wheat	rye	Conv-OA	2	0.75	0.87	0.00	0.00	3	3
A8	193	365	183	5.5	20	1.5	wheat	mustard	CA	7	0.09	0.03	0.17	0.19	0	0
A9	205	404	188	6.8	18	1.4	wheat	rapeseed	Conv	20	0.89	0.78	0.42	0.81	0	0
A10	221	453	49	7.1	20	1.3	wheat	alfalfa	Conv	20	0.37	0.13	1.00	0.94	0	0
A11	256	351	117	7.4	28	1.2	wheat	cover	OA	20	0.83	0.70	0.00	0.00	6	6
A12	223	350	165	7.3	23	1.3	wheat	cover	Conv	20	0.55	0.53	0.58	0.63	2	2
A13	250	411	73	6.9	30	1.3	wheat	rye, lentil	CA-OA	2	0.31	0.28	0.00	0.06	2	3
A14	180	383	121	6.8	29	1.2	wheat	cover	Conv-CA	3	0.06	0.03	0.75	0.50	1	1
A15	194	394	90	6.8	26	1.4	wheat	wheat	Conv-CA	3	0.09	0.06	0.75	0.81	2	2
A16	293	337	67	8.1	23	1.4	wheat	mustard	Conv	20	0.38	0.25	0.92	0.69	2	2
A17	228	350	198	8.1	34	1.3	wheat	rapeseed	CA	10	0.00	0.00	0.92	0.81	1	1
A18	195	454	69	6.9	24	1.3	wheat	bare	Conv-OA	3	0.68	0.51	0.00	0.00	3	3
A19	280	266	261	7.7	26	1.3	wheat	clover	Conv-OA	2	0.49	0.53	0.00	0.00	4	4
A20	202	407	51	7.3	19	1.4	wheat	cover	CA-OA	2	0.69	0.52	0.00	0.00	2	2
A21	336	226	244	8.0	31	1.3	wheat	barley	Conv	20	0.38	0.52	0.50	0.63	0	0

Conv: conventional agriculture, CA: conservation agriculture, OA: organic agriculture, Conv-CA: transition from conventional to conservation (≤ 3 years), Conv-OA: transition from conventional to organic (≤ 3 years), CA-OA: transition from conservation to organic (≤ 3 years), SOM: soil organic matter, BD: bulk density.

4.2.3 Soil and biodiversity sampling

Samples were collected in the 21 plots in autumn 2020 and 2021, with three replicates per plot spaced by 25 m and located at 25 m of the field margins (cf. General introduction).

4.2.3.1 Soil

For each replicate, the soil was sampled at 0-10 and 10-20 cm depth by mixing eight soil cores extracted with an auger. In 2020, soil was sieved at 4 mm, air dried and sent for analysis of physico-chemical characteristics at the INRAE Laboratory of Arras. In addition, soil bulk density was measured at 0-10 and 10-20 cm depth by extracting soil cores (10 cm in diameter, 10 cm high), that were then dried (105°C, 48h) and weighed.

4.2.3.2 Microorganisms

A fraction of soils sampled at 0-10 and 10-20 cm depth was sieved at 4 mm, lyophilized and kept at -30°C for molecular analyses at the GenoSol platform (INRAE, Dijon, France). Microbial DNA was extracted from 1g of soil following the protocol of Plassart et al. (2012), purified using a NucleoSpin Soil kit (Macherey-Nagel) and quantified by a fluorometric assay. Bacterial and fungal densities were estimated by real-time quantitative polymerase chain reaction (qPCR) of the 16S and 18S rRNA genes using a thermal cycler apparatus (StepOne Plus 96, Applied Biosystems®).

4.2.3.3 Meso- and macrofauna

Mesofauna was collected using small soil cores (5 cm in diameter, 4 cm high) and extracted using a MacFayden extractor. The extraction was performed during eight days with a progressive increase in temperature from 25°C to 45°C. Collembola, Acari and other mesofauna representatives were sorted and counted under binoculars.

Macrofauna, including macroarthropods, gastropods and earthworms, was hand-sorted from 25 x 25 x 25 cm soil blocks directly on site, and identified at the order, class or family level under binoculars.

Trophic groups of soil fauna and potential trophic relations were determined following the recent review of Potapov et al. (2022). The following soil fauna trophic groups were considered in our study: detritivores, predators, plant feeders, omnivores and earthworms (e.g. feeding on plant residues and soil).

4.2.4 Statistical analyses

The three replicates were averaged to obtain a representative value per plot. Fauna density was obtained by dividing abundance data by the area of soil extraction (0.0025 m² for mesofauna, 0.625 m² for macrofauna). The normality and homogeneity of variances were tested with a Shapiro-Wilk test ($\alpha > 0.05$) and a Bartlett test ($\alpha > 0.05$).

The effects of cropping systems on soil properties and organisms were assessed using respectively an analysis of variance (ANOVA) or generalized linear models (GLM) with a quasi-Poisson family to account for the non-normal distribution and overdispersion of data. A Tukey HSD post-hoc test was performed to assess differences between systems using the R package *multcomp* (Hothorn et al., 2022).

Effect sizes and 95% confidence intervals were computed with the Hedges' g estimate, accounting for small sample size, and the R package *effsize* (Torchiano, 2020). Effect sizes were assessed to compare the density of soil organisms under alternative *versus* conventional systems and transitioning *versus* initial systems. An effect size was computed for each group of soil organisms using data collected both in 2020 and in 2021. Microbial biomass, bacterial and fungal densities were averaged at 0-20 cm depth. A mean effect size was calculated based on the effect sizes of all groups and a fixed effect model using the R package *meta* (Schwarzer, 2007). A positive effect size indicates that the observed density is higher in the alternative than in the conventional system or in the transitioning than in the initial system. An effect size is significant if its confidence interval does not include zero. A rule of the thumb is that the Hedges' g estimate is large if $g > 0.8$, medium if $0.8 > g > 0.5$, small if $0.5 > g > 0.2$ and negligible if $g < 0.2$ (Hedges and Olkin, 1985).

A path analysis, which is a type of structural equation modeling (SEM), was conducted to assess the relations between practice intensity, soil organic matter (SOM) and trophic groups of soil organisms using the R package *lavaan* (Rosseel, 2012). This analysis relies on the data collected in 2020 and 2021, which were normalized using z-transformation. The variables included in the model are presented in Table 4.2. SOM, bacterial and fungal abundances correspond to the average value of results obtained at 0-10 and 10-20 cm depth in order to limit the number of variables. We did not include plant feeders due to their low density, nor further soil properties, even if it could have been relevant, as only a limited number of variables can be included in the model owing to the limited number of samples. A biologically relevant model was constructed to assess the causal relations between Itill, Itreat, nbOrg, SOM, bacteria, fungi, mesofauna detritivores, mesofauna predators, macrofauna detritivores, macrofauna predators, macrofauna omnivores and earthworms (Appendix S4.1). This model was simplified by the stepwise exclusion of non-significant relations, starting from the highest *P*-values, until we obtained an adequate model (Appendix S4.1). Model adequacy was determined by looking at the

difference between predicted and observed covariance matrices (χ^2 -test, $P > 0.05$), the root mean squared error of approximation index (RMSEA < 0.1) and the comparative fit index (CFI > 0.9) (Grace, 2006).

All the statistical analyses were performed using R software version 3.6.3 (R Development Core Team 2020).

Table 4. 2 Variables selected for the model used in path analysis (i.e. SEM). Mean densities and attribution of trophic groups of soil organisms are reported in Table S4.1, S4.2 and S4.3. Values regarding SOM are reported in Table 4.1.

Variable	Description
(a) Practice intensity	
Itill	Tillage intensity index
Itreat	Pesticide treatment intensity index
nbOrg	Number of organic matter inputs over 5 years
(b) Soil properties	
SOM	Soil organic matter content averaged at 0-20 cm depth
(c) Trophic groups	
Q16S	Bacterial abundance (10^9 16S copies.g soil ⁻¹) averaged at 0-20 cm depth
Q18S	Fungal abundance (10^8 18S copies.g soil ⁻¹) averaged at 0-20 cm depth
Macrofauna detritivores	Dermestidae, Diplopoda (Julida and Polydesmida), Isopoda
Mesofauna detritivores	Collembola, Oribatida
Earthworms	Endogeic earthworms are mostly soil feeders whereas epigeic and anecic earthworms are litter feeders, but most earthworms species are opportunistic
Macrofauna omnivores	Coleoptera larvae, Dermaptera, Diptera larvae, Elateridae
Macrofauna predators	Araneae, Carabidae, Chilopoda (Geophilomorpha, Lithobiomorpha), Diplura, Staphylinidae
Mesofauna predators	Actinidida, Gamasida

4.3 Results

4.3.1 Soil physico-chemical properties and practice intensity

Soil properties were not significantly different between cropping systems except for a higher K and Mg content (ANOVA, $P < 0.05$) in OA than in Conv-CA systems at 10-20 cm depth (cf. General introduction, Tables I.5 and I.6). The SOM content tends to be higher in CA at 0-10 cm depth and in OA at 10-20 cm. Overall, SOM was concentrated at the surface in CA and homogeneously mixed in soil in OA.

Tillage intensity was very low in conservation plots, and did not differ significantly between conventional and organic systems (cf. General introduction, Figure I.10). Pesticide treatments were absent in organic plots, and pesticide treatment intensity varied within conventional and conservation plots with no significant differences between these two systems. More organic matter inputs were recorded in OA and Conv-OA than in Conv, CA and Conv-CA. An increase in the number of organic matter inputs was related to an increase in SOM at 10-20 cm depth (cf. General introduction, Table I.7).

4.3.2 Effects of cropping systems on soil organisms

4.3.2.1 Microorganisms

Conv-CA had a significant and large negative effect ($ES = -1.19$) on fungal abundance at 10-20 cm depth (Figure S4.1). We observed no significant effect on mean molecular microbial biomass, bacterial abundance and fungal abundance at 0-20 cm depth (Figure 4.1). However, compared to Conv, OA tended to have a large positive effect on all microbial variables, whereas CA had no effect on bacteria and inversely tended to have a negative effect on fungi (Figure 4.1).

4.3.2.2 Meso- and macrofauna

CA had a significant and large positive effect ($ES = 1.10$) on Collembola density compared to Conv (Figure 4.1). Conversely, OA had a non-significant and lower effect than CA on Collembola. In addition, CA-OA had a large but non-significant negative effect on Collembola density compared to CA.

Both CA and Conv-CA had a significant and large positive effect (2.44 and 1.43 respectively) on the density of Coleoptera larvae, reflecting total Coleoptera density (Figure 4.1). CA also had a significant and large positive effect (1.25) on Gastropoda density with a similar trend observed for Conv-CA (Figure 4.1). In addition, both Conv-CA and Conv-OA had a significant and large positive effect (1.33 and 1.09 respectively) on earthworm density (Figure 4.1). CA-OA systems had a significant large negative effect (-1.63) on Araneae density (Figure 4.1). Furthermore, CA tended to have larger effects than OA on the density of Araneae, Chilopoda, Coleoptera, Gastropoda and earthworms, whereas OA tended to have a larger and positive effect on the density of Diplopoda (Figure 4.1).

4.3.2.3 Overall effect

A significant and positive mean effect on the density of soil organisms was observed for several alternative systems with in decreasing order CA ($ES = 0.39$, $95\%CI = [0.13; 0.65]$), Conv-CA (0.34 [0.08; 0.60]) and OA (0.28 [0.03; 0.54]). Inversely, CA-OA showed a significant and negative mean effect (-0.45 [-0.76; -0.14]) and Conv-OA had a negative but non-significant effect (-0.15 [-0.41; 0.10]).

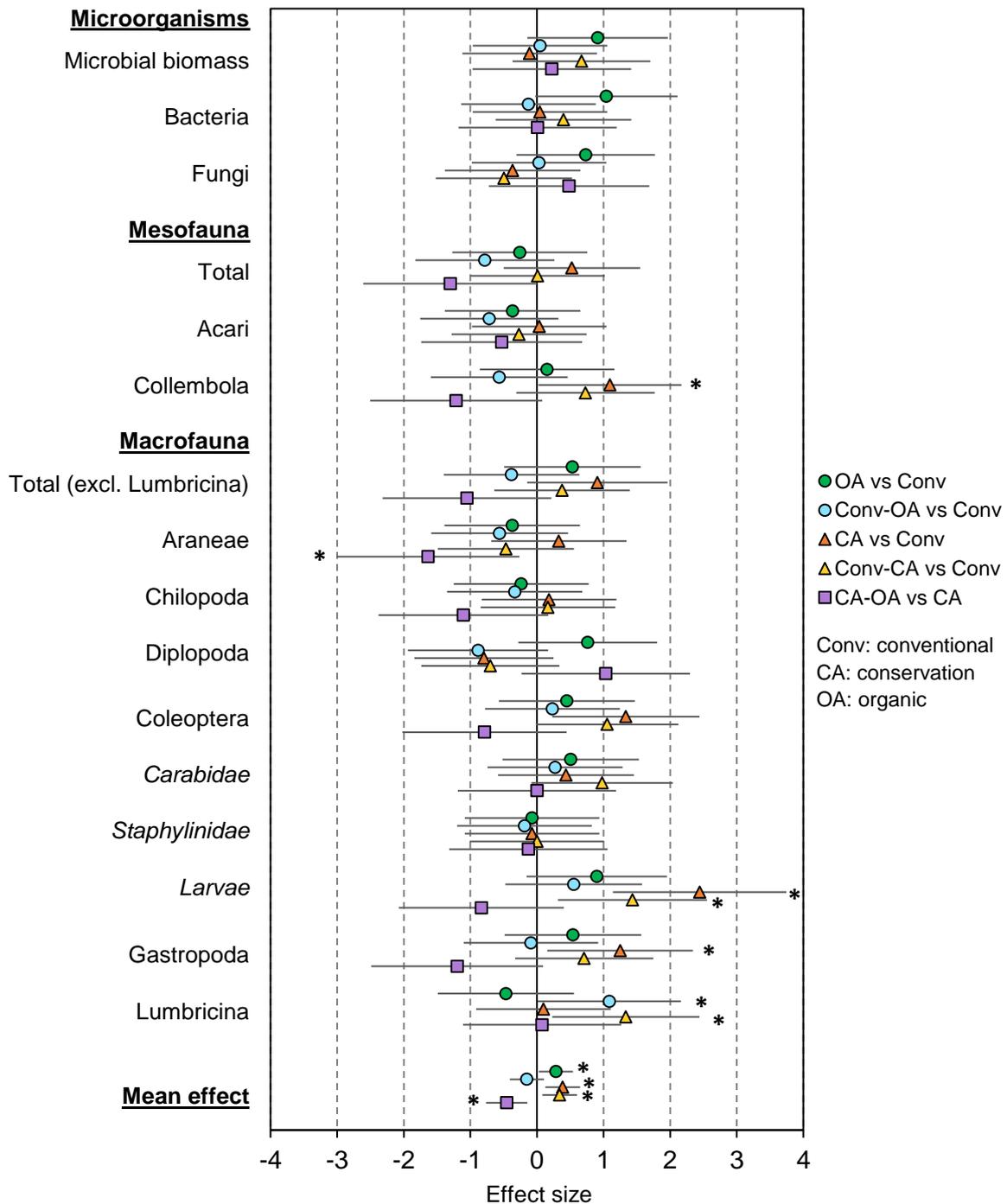


Figure 4. 1 Effect sizes (Hedges' g estimate and 95% confidence interval) of stable or recent alternative cropping systems on the density of soil microorganisms, mesofauna and macrofauna, and mean effect on the density of soil organisms. Effect sizes are based on the density of soil organisms collected in 2020 and 2021. Symbols represent the effect size obtained for the comparison between two systems (green circles = stable organic *versus* conventional, blue circles = recent organic *versus* conventional, orange triangles = stable conservation *versus* conventional, yellow triangles = recent conservation *versus* conventional, purple squares = recent organic from conservation *versus* stable conservation). Effect sizes are significantly positive or negative (*) when the confidence interval does not include zero.

4.3.3 Path analysis

The path analysis (i.e. SEM) demonstrated that cropping practices, SOM and trophic groups of soil organisms interact through multiple paths in agricultural soils (Figure 4.2 and Table S4.4). Tillage intensity had a negative direct effect on SOM and on the density of mesofauna detritivores, omnivores and in a lesser extent on earthworms (Figure 4.2). Mesofauna detritivores in turn had a direct positive effect on mesofauna and macrofauna predators (Figure 4.2). Organic matter inputs had a positive effect on macrofauna detritivores and SOM, which in turn positively affected bacteria (Figure 4.2). Pesticide treatment intensity had a low but positive relation with macrofauna detritivores and predators (Figure 4.2). In addition, the density of mesofauna detritivores was positively related to the density of fungi and negatively to the density of earthworms (Figure 4.2).

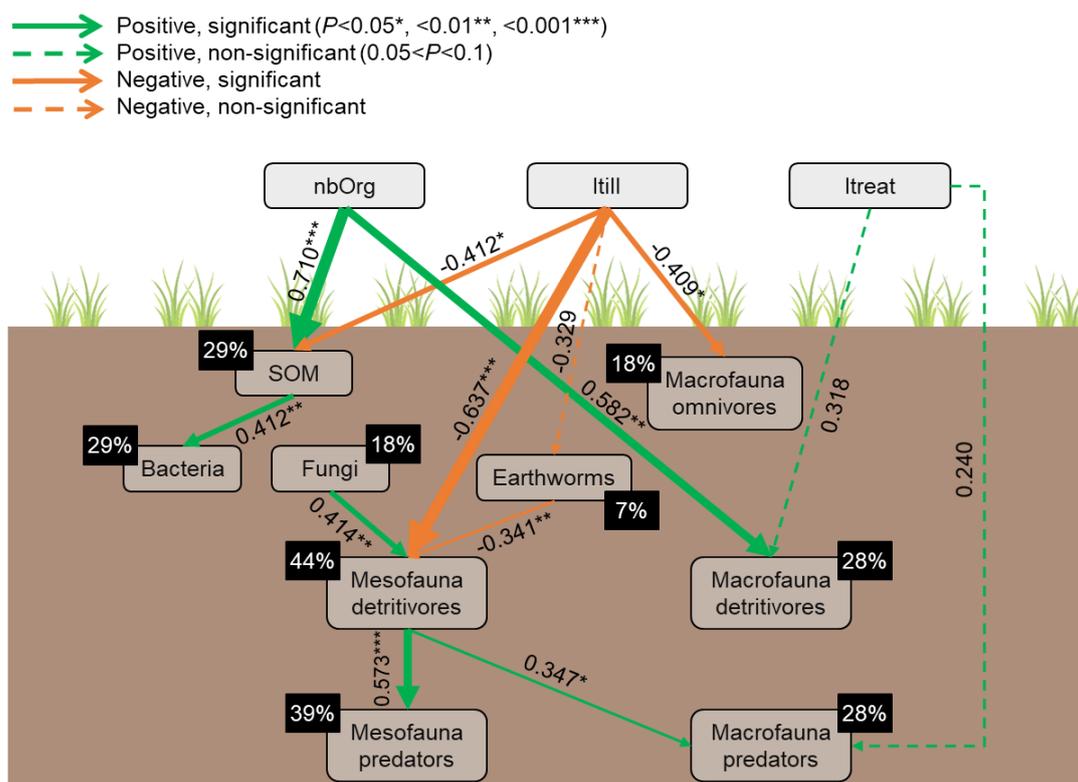


Figure 4. 2 Path analysis representing the relations between tillage intensity (ltill), pesticide treatment intensity (ltreat), number of organic matter inputs over 5 years (nbOrg), soil organic matter content (SOM) and density of soil organisms (bacteria, fungi, mesofauna detritivores and predators, macrofauna detritivores, predators and omnivores, earthworms) in cropping systems ($\chi^2 = 40.67$, $df = 33$, $P = 0.17$; $RMSEA < 0.08$; $CFI > 0.9$). Numbers associated to arrows are standardized parameter estimates with their significance ($P < 0.05^*$, $< 0.01^{**}$, $< 0.001^{***}$). Percentages of explained variances are reported in black boxes next to variables.

4.4 Discussion

Our results demonstrate that stable and transitional alternative systems have a major influence on the soil community. Changes in soil properties and direct effects of individual practices affect the density of several groups of soil organisms with cascading effects through trophic interactions. Therefore, this study highlights the need to account for multiple groups of soil organisms and for their interactions when assessing the effect of cropping systems on soil biodiversity.

4.4.1 Overall effect of cropping systems on the density of soil organisms

The density of soil organisms is highly variable within cropping systems because of the diversity of applied practices and differences in soil properties. However, over the two years of our study, stable alternative systems and systems in transition demonstrated some important effects on the density of soil organisms.

Our results showed that conservation systems promoted the density of several taxa compared to conventional systems, both in the first years of transition and at a stable stage. The higher density of Collembola and Coleoptera larvae that we observed can be explained by the absence of tillage (Holland and Reynolds, 2003; Coulibaly et al., 2017). In our study, Coleoptera larvae were mostly Carabidae larvae. Their higher density suggests a higher Carabidae density, as observed during one of the year of our study, which also benefits from lower tillage intensity (Kromp, 1999). On the other hand, stable conservation systems also promoted Gastropoda density (i.e. mostly slugs) that are considered as pests in cropping systems as they cause severe damage to crops. This is in line with previous studies reporting a potential increase in pest species in conservation systems (Scopel et al., 2013; Chabert and Sarthou, 2020). However, the observed increase in Coleoptera larvae indicates a potential for biological regulation (Kromp, 1999).

Organic systems had lower effects on the soil community than conservation systems with few and non-significant differences compared to conventional agriculture. Similarly, organic systems were reported to have variable effects on the density of soil organisms depending on taxa and on the context (Bengtsson et al., 2005; Hole et al., 2005) with reported negative results due to intensive tillage (Patterson et al., 2019). It is however noteworthy that stable organic systems tended to promote the density of microorganisms (mostly bacteria) compared to conventional agriculture, as reported in previous studies (Hole et al., 2005; Lori et al., 2017). Besides, organic agriculture may foster the diversity more than the density of soil organisms (Bengtsson et al., 2005; Schrader et al., 2006).

Overall, both conservation and organic agriculture benefited the density of soil organisms compared to conventional agriculture. Conservation systems have a higher mean effect on the density of soil organisms than organic ones, but the difference is not significant. These two alternative systems may differ mostly as they promote different groups of soil organisms. Compared to organic systems, conservation systems can have higher and more significant benefits on macrofauna groups (e.g. Coleoptera larvae) and are the only system to promote Collembola. Inversely, organic systems tend to have a positive effect on microorganisms, which was not observed for conservation systems, and could benefit other macrofauna groups (e.g. Diplopoda), but these effects were lower and more variable than effects observed for conservation systems.

Systems in transition toward organic or conservation agriculture showed effects that were not observed in stable systems. The transition to conservation agriculture affects the fungal abundance at 10-20 cm depth possibly due to changes in the community composition, which was reportedly related to the conversion to no-tillage and associated changes in soil parameters (Stubbs et al., 2004; Zhang et al., 2022). In addition, systems in transition from conventional to organic and to conservation agriculture both showed an increase in earthworm density, but this effect seems to be transitory. In agreement with this, earthworm communities were reported to have a long response time to changes in cropping practices (Pelosi et al., 2016). In contrast, systems in transition from conservation to organic agriculture had a significant negative effect on the density of Araneae and tended to have a low density of several other groups of soil fauna (e.g. Collembola, Chilopoda, Coleoptera larvae). Conservation and organic systems affect soil in different ways (i.e. chemical *versus* physical disturbances), implying major differences in the soil functioning and in the soil community composition. Unlike in conventional systems, a large part of species in conservation systems may not be adapted to physical disturbances. Therefore, a transition from conservation to organic agriculture, which involves the onset of tillage, could lead to larger changes in the soil community composition than a conversion from conventional agriculture.

4.4.2 Influence of applied practices on soil trophic interactions

The intensity of applied practices within cropping systems affects the density of soil organisms as well as their trophic interactions. In our model, tillage intensity influenced the highest number of soil biological variables, with a large direct negative effect on the density of mesofauna detritivores, intermediate direct effects on SOM and on the density of omnivores, and a smaller non-significant effect on earthworms. A high tillage intensity results in the homogenization of organic matter distribution in soil, as observed here in organic systems, improving its accessibility to microorganisms that in turn accelerate its degradation (Stubbs et al.,

2004). This increased decomposition, and a lower physical protection of SOM in aggregates under intensive tillage, can lead on the long-term to a depletion of SOM (Balesdent et al., 2000). Furthermore, negative effects of tillage are widely reported for soil fauna such as earthworms and mesofauna detritivores (Wardle, 1995; van Capelle et al., 2012). Tillage can cause direct physical damage to soil fauna and indirect effects through the modification of the soil habitat and changes in the food resources distribution (Kladivko, 2001).

In our study, as tillage increases, the lower density of mesofauna detritivores has cascading effects on the density of mesofauna and macrofauna predators that feed on them, and inversely benefits the abundance of the fungi they feed on. Therefore, mesofauna detritivores seem to have a central place in the soil food web of the studied agroecosystems. Similarly, Collembola are reported to feed preferentially on fungi (Filser, 2002) and to be common preys of macrofauna predators in agricultural soils such as carabids (Bilde et al., 2000). Furthermore, we expected earthworms to have a positive effect on mesofauna detritivores through changes in the soil habitat (i.e. cast, burrows) (Wickenbrock and Heisler, 1997; Wardle, 2006; Birkhofer et al., 2011). Yet, we found earthworms to exert a negative effect on mesofauna detritivores, in contrast to previous findings, but for which we are unable to provide a reliable explanation. Indeed, this negative effect could result from a competition for organic matter, but this was only reported for invasive earthworm species (Jochum et al., 2022).

Conversely, omnivores were not related to other components of the soil food web. Omnivory is well represented in soils and was often reported to weaken soil trophic cascades (Kulmatiski et al., 2014). However, omnivorous organisms could be main drivers of pest regulation through their capacity to feed on the available resource (i.e. flourishing pest species) and hence benefit crop growth (Kulmatiski et al., 2014).

Organic fertilization logically had a large positive effect on SOM with cascading effects on bacteria, as reported in previous studies (Treonis et al., 2010). It also has a large direct positive effect on macrofauna detritivores as they can feed on coarse organic matter (Ponge et al., 2013). Yet, the lack of relation between bacteria, macrofauna detritivores and other compartments of the soil food web do not indicate cascading effects on the rest of the soil food web. The isolated aspect of bacteria may be simply related to the absence of microfauna representatives (i.e. nematodes, rotifers) in our model, which are the main consumers of bacteria and are in turn consumed by mesofauna (Potapov et al., 2022). Using SEM, previous studies found significant relations between soil microbial biomass and different trophic groups of nematodes (Eisenhauer et al., 2013; Sun et al., 2022). In addition, the absence of relation between fungi and organic matter inputs could be related to the type of organic matter. Furthermore, macrofauna detritivores were mostly represented by diplopods that are reported to be poorly related to other trophic groups and to

serves as preys only for specialized predators (Forthman and Weirauch, 2012; Potapov et al., 2022).

Pesticide treatment intensity was observed to be a second order factor of influence on the soil food web, with limited effects on macrofauna detritivores and predators. Macrofauna predators may be more impacted by tillage, through their trophic link with mesofauna, than by pesticide treatments. However, our results run counter to previous studies reporting an overall negative effect of pesticides on macrofauna detritivores (Domínguez et al., 2014; Pearsons and Tooker, 2021) and a negative effect of insecticides on macrofauna predators density in cropping systems (Lu et al., 2022). The observed increase in the density of macrofauna detritivores may be explained by an increase in dead weed biomass following herbicide application. However, macrofauna detritivores were almost only comprised of diplopods for which we observed a negative effect of pesticide treatments (i.e. increased density in organic systems). Therefore, the effect of pesticide treatments on macrofauna detritivores remains ambiguous.

All trophic groups were impacted by the intensity of practices except for fungi. As we showed that fungi are impacted by cropping systems, in particular by conservation systems at 10-20 cm depth, we suggest that their relation to practices may be more complex or group dependent. For instance, some groups of fungi, such as arbuscular mycorrhizal fungi, were reported to be especially sensitive to tillage (Jansa et al., 2003).

Furthermore, our model poorly explained the variability in earthworm density (7%). This suggests that one or several variables not included in our model may have more effects on earthworms than practice intensity and other trophic groups. In particular, earthworm density is reported to respond to soil bulk density (Beylich et al., 2010) and pH (Mele and Carter, 1999), which are indirectly related to applied practices in cropping systems. Soil properties were not included in our model due to limitation in the number of variables that can be realistically used in a model, but they do take part in the effect of practices on soil organisms. Indeed, tillage was also demonstrated to influence indirectly the diversity and the composition of bacterial communities through its effect on soil organic carbon and bulk density (Li et al., 2021). Overall, soil is a complex habitat whose properties are driving trophic interactions between organisms (Erktan et al., 2020). However, very large datasets will be required to assess the effects of practices on soil properties and on multiple groups of soil organisms within the same model.

4.4.3 Inputs to current research on alternative cropping systems and perspectives

Our study showed that both conservation and organic agriculture can benefit the density of soil organisms under real conditions, with a tendency for a larger positive effect of conservation agriculture. This confirms results observed previously in one experimental trial (Henneron et al.,

2015). Furthermore, we assessed the effect of alternative systems on soil mesofauna, which was poorly investigated compared to other groups of soil organisms (Christel et al., 2021), and showed that Collembola in particular benefit from conservation agriculture.

We showed that strong positive or negative effects can be observed on the density of soil organisms during the transition toward alternative systems, but that these changes might be transitional for some taxa (i.e. not observed on stable systems). In addition, we highlighted that the conversion from conservation to organic agriculture could have strong negative effects on soil biodiversity during the first years of transition due to important changes in the applied practices. As there is currently a research gap on the consequences of the transition from conservation to organic systems, further studies are required to assess if the effects observed in our study are transient or if they imply a long recovery time. All of this reinforces the need for long-term studies on the effects of alternative systems, since conversion and with regular samplings in order to assess the time required for a stabilization of the soil community, as advocated in previous studies (Pelosi et al., 2015).

This study also illustrates the effects of practice intensity on the soil food web. Our results support the fact that tillage is probably the main factor of influence for numerous groups of soil organisms, and further demonstrate its effects on the soil food web. In addition, we suggest that mesofauna detritivores have a central place in the agricultural soil food web as they present the highest number of relations with other groups. These results were obtained by using SEM, further demonstrating the usefulness of this approach for soil ecology (Eisenhauer et al., 2015).

Future studies should pursue the investigation on cropping systems and practices by assessing the interaction between soil organisms under different contexts. In addition, it is necessary to better understand the link between belowground and aboveground biodiversity to have an overview of the effect of cropping systems on the overall biodiversity (Wardle, 2006; Eisenhauer and Schädler, 2011).

4.5 Conclusion

In this study, we demonstrate that stable and transitional alternative systems have a significant influence on the density of several groups of soil organisms. Conservation and organic systems both have a positive effect on the overall density of soil organisms compared to conventional systems and this effect tends to be higher for conservation systems. The influence of cropping systems on soil biodiversity is further explained by observing the effects of practice intensity on the soil food web. First, we showed that the benefits of conservation and organic agriculture may be attributed to no-tillage and organic matter inputs respectively, and were poorly related to pesticide treatments. Tillage was observed to have both direct effects on soil

organisms and cascading effects across the soil food web. Organic matter inputs benefited to bacteria and macrofauna detritivores, both of which tended to be higher under organic systems. Furthermore, we observed that mesofauna detritivores have a central place in the soil food web of agroecosystems due to their large density and their high number of interactions with other groups. However, interactions between soil trophic groups are conditioned by the applied practices. The observed differences in the density of soil organisms and in their interactions may provide insight on the functions they maintain in soil, and support the fact that different cropping systems may promote different ecosystem services. Overall, this study highlights the need to account for multiple groups of soil organisms and for their interactions when assessing the effects of cropping systems on soil biodiversity. Further investigations could help improving our understanding of the full range of disturbances caused by agricultural practices and to promote systems that efficiently rely on soil biodiversity and associated soil functions.

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Transition toward the chapters 5 and 6

In the chapters 1 to 4, we demonstrated that cropping systems and individual practices influence the density and diversity of various soil organisms as well as their interactions. Soil organisms play a major role in soil functioning through their activities (Figure T.2). Therefore, the influence of cropping systems on the soil community may affect the soil functions with important consequences for the provision of ecosystems services by agricultural soils. Despite a growing research interest, the links between biodiversity and ecosystem functioning remain poorly understood. It is not possible to infer with certainty the consequences of a change in the density or diversity of soil organisms on the soil functioning. The assessment of the effects of cropping systems on soil functioning requires a direct assessment of soil functions. In addition, in order to understand the importance of the biodiversity for the functioning of ecosystems, it is essential to focus on various functions performed by various soil organisms (Gamfeldt et al., 2008). In the following two chapters, we investigate several soil functions maintained by soil organisms to understand the effect of cropping systems on the soil functioning. In the chapter 5, we investigate the role of soil organisms, and in particular of soil fauna, on litter decomposition through a meta-analysis on litterbag experiments conducted in agricultural soils. Then, in the chapter 6, we present results of measurements performed to assess litter decomposition, C and N mineralization and soil aggregate stability.



Figure T. 2 Illustration of activities performed by soil organisms influencing soil functions. From left to right: a millipede (Diplopoda class, Polydesmida order) on fecal pellets produced from the consumption of plant debris; an earthworm's burrow within a soil clod collected in a wheat field; fungal hyphae growing on maize residues extracted from a litterbag buried in soil for five months. Photo credit: J. Chassain.

———— Chapter 5 ————

Role of different size classes of organisms in cropped
soils: what do litterbag experiments tell us?

A meta-analysis.

Juliette Chassain¹, Laure Vieublé Gonod¹, Claire Chenu¹, Sophie Joimel¹

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¹ Université Paris-Saclay, INRAE, AgroParisTech, UMR ECOSYS, 91120, Palaiseau, France

Abstract

Soil organisms are essential for the functioning of agrosystems, especially in the process of litter decomposition. Litterbags constitute one common way to assess litter decomposition and to investigate the role of the different groups of soil organisms in the decay activity. However, there is currently no standardized litterbag protocol to measure the effects of soil organisms on litter decomposition. Furthermore, litterbag studies remain scarce in agrosystems and little information is available about the influence of different groups of soil organisms depending on agricultural practices. The development of cropping systems that rely on high soil biodiversity and fertility however requires a detailed understanding of these processes. In order to address this need and to have an overview of the existing protocols, we conducted a review on litterbag experiments in annually cropped soils. We collected information on the experimental design (e.g. duration, number of replicates), litterbags (e.g. size, mesh), climate, soil type, standing crop, enclosed litter (e.g. litter type, quality) and methods used to characterize organic matter decomposition and soil organisms. The effects of soil organisms of different size classes (meso- and macrofauna) was assessed with a meta-analysis performed on studies using litterbags of different mesh sizes. The general effect size of soil macrofauna, mesofauna (in addition to soil microorganisms) and of their combination was assessed with a three-level random-effect model accounting for the random effect at the study level. This effect was compared for subgroups based on climate, soil, standing crop, agricultural system and litter type as categorical factors, and for depth of bags, duration of the experiment and size of litter pieces as continuous factors. Macrofauna, mesofauna and the combination of both were found to significantly increase litter decomposition. Surprisingly, meso- and macrofauna contributed equally to litter decomposition and their effects were not additive (when comparing the role of meso- and macrofauna independently and simultaneously). These effects tend to be influenced by various factors: climate, soil, standing crop and agricultural system for macrofauna; standing crop and litter type for mesofauna; and soil, standing crop and litter type for their combined effects. Multi-mesh litterbag experiments showed that even in soils with high disturbances, soil organisms of several size classes have a significant impact on organic matter decomposition. While this study showed that both soil macrofauna and mesofauna increased litter decomposition in annual cropping systems, there are still numerous gaps in our knowledge of the impacts of the agronomic (e.g. cropping system, practices, crop type) and environmental contexts (e.g. climates, soils). Forecasting future studies, we provide guidelines to develop a standard litterbag protocol adapted to the specificities of annually cropped soils.

5.1 Introduction

Organic matter plays a key role in soil functioning and in particular on soil fertility through the release of nutrients during decomposition (Tiessen et al., 1994; Carter, 2002). In cropping systems, organic matter comes from the restitution of crop residues to the soil, inputs of organic waste products (e.g. livestock manure or compost) or burial of cover crops. Its distribution in soil depends on agricultural practices. In particular, soil tillage leads to a more or less homogenous soil-organic matter mixture over variable depths (Salinas-Garcia et al., 1997; Kay and VandenBygaart, 2002).

Soil organisms are essential drivers of the decomposition of organic matter. According to their size, microorganisms (< 0.1 mm), mesofauna (0.1 - 2 mm) and macrofauna (> 2 mm) (Swift et al., 1979) can act at different spatial and temporal scales. However, the contribution and context specific effects of different groups of soil organisms during decomposition is still not well known. In addition, the density and diversity of soil organisms are generally lower in agrosystems than in any other ecosystem (Tsiafouli et al., 2015; Joimel et al., 2017). Intensive management of cropping systems affects soil quality in the short and long term by modifying physical, chemical and biological soil characteristics, with the intensity of changes depending on pedoclimatic conditions and farming practices (Hati et al., 2007; Mazzoncini et al., 2010). These changes can strongly affect decomposition, thus threatening core ecosystem functions such as nutrient cycling (Bradford et al., 2002; Chen et al., 2020). A detailed understanding of the role of soil organisms during organic matter decomposition is fundamental to develop agroecology and to implement systems that will rely on soil biodiversity and fertility.

To address the gaps on the role of soil organisms on decomposition, numerous studies were conducted under laboratory conditions (Huhta, 2007). However, these experiments showed limitations as they can present lower diversity than in field conditions (Kardol et al., 2016). Consequently, the simulated biodiversity and modification of soil properties are not representative of the reality, which could be addressed by studies *in situ*.

Litterbags and teabags constitute one common way to assess decomposition *in situ* and over a long period of time (Didion et al., 2016). The litterbag technique was developed in the middle of the 19th century (Crossley and Hoglund, 1962; Edwards and Heath, 1963), while the teabag method was introduced recently (Keuskamp et al., 2013). The advantage of litterbags is that they contain organic matter that can be adapted to the context of the experiment. In addition, litterbags can be used to study the contribution of different groups of soil organisms to litter decomposition by selectively excluding some fauna size classes. This selection can occur either by using different mesh sizes in order to physically exclude some organisms, or by a chemical treatment inhibiting part or all of the organisms. The exclusion of organisms by mesh size has sometimes been

criticized due to a potential effect of different mesh sizes on decomposition (Anderson, 1973; Bradford et al., 2002), but this technique was approved as a reliable method to quantify the role of soil organisms in litter mass loss (Seastedt, 1984; Bokhorst and Wardle, 2013). However, contrary to the teabag method, no standardized method is currently available for one who wants to start a litterbag experiment. Yet litterbags mimic surface or buried crop residues in cropping systems and therefore allow looking at the main factors affecting their decomposition. Forecasting future studies on soil biodiversity in agricultural context, a standard protocol adapted to the specificities of annually cropped soils could be beneficial.

The litterbag method has been subjected to several reviews and meta-analyses, focusing on the history of the method (Huhta, 2007) and on the impacts of several factors on litter decomposition, namely soil fauna (Seastedt, 1984; Kampichler and Bruckner, 2009; Frouz et al., 2015; Zhang et al., 2015; González and Lodge, 2017; Frouz, 2018), climate and litter quality (García-Palacios et al., 2013; Zhang et al., 2015; Krishna and Mohan, 2017), nitrogen addition (Knorr et al., 2005) and pesticides (Knacker et al., 2003). In a first review, Seastedt (1984) estimated microarthropods to increase litter decomposition by 23%. The meta-analysis of Kampichler and Bruckner (2009) confirmed the positive effect of microarthropods over several land-use types, notably in agricultural soils. In a later meta-analysis, Frouz et al. (2015) found macrofauna to significantly increase litter removal but not litter mineralization. García-Palacios et al. (2013) and Zhang et al. (2015) estimated that exclusion of all fauna from litterbags, by physical or chemical methods, decreased litter decomposition by 27% and 35% respectively. Recently, Huang et al. (2020) found that high diversity of earthworm functional groups increased litter and soil organic carbon decay in tree plantation and forests. In forest ecosystems, soil detritivores were reported to strongly increase leaf litter and wood debris decomposition, with macrofauna or mesofauna having a larger impact depending on the forest type and climatic conditions (González and Lodge, 2017; Krishna and Mohan, 2017). Previous reviews were mostly focused on forest ecosystems or grasslands (González and Lodge, 2017; Krishna and Mohan, 2017), while studies on cropped soils remain scarce (Knacker et al., 2003; Kampichler and Bruckner, 2009). Furthermore, none of these reviews or meta-analyses investigated the comparative effect of different size classes of organisms. In this context, we provide here the first study to assess litter decomposition for several size classes of soil organisms in annually cropped soils.

The objectives of this study were (1) to assess the effects of different size classes of soil organisms on litter decomposition in annually cropped soils through the litterbag method and (2) to provide an overview of the existing protocols of the litterbag method in cropping systems. To this end, we conducted a meta-analysis of litterbag experiments in annually cropped soils. In

particular, we aimed to quantify the effects of soil organisms of different size classes (meso- and macrofauna) in studies using bags of different mesh sizes.

5.2 Materials and methods

5.2.1 Data collection

Literature was surveyed in November 2019 for studies assessing the effect of soil organisms on litter decomposition with the litterbag method. We searched in all the Web of Science databases, with no restriction on the publication year, for papers containing the keywords “litter”, “decomposition, degradation, decay or breakdown”, “*fauna, *organism*, animal*, *flora, *arthropod*, *invertebrate*, collembola*, springtail*, earthworm*, lombric*, fungi, bacter* or nematod*”, “*bag*” and “soil*”. In addition, we deliberately excluded papers containing the words “bagasse*”, “stream*”, “marine” and “aquatic”. The search returned 1040 results. Further studies were added by checking articles included in previous reviews (Knacker et al., 2003; Kampichler and Bruckner, 2009; García-Palacios et al., 2013; Frouz et al., 2015; Zhang et al., 2015). In total, 1106 articles were collected through the different search paths. Studies had to meet the following criteria to be selected:

- they should discuss the decomposition of organic matter or nutrient loss using the litterbag method and provide sufficient information about the protocol (e.g. mesh size) ;
- they should focus on agricultural soils, more precisely on annually cropped soils, but not on agroforestry, orchards, vineyards, grasslands, fallows, bare soils and field margins ;
- they should present data on organic matter decomposition (e.g. mass loss, decomposition rate), and not focus on a specific component only (e.g. lignin) ;
- they should use only physical methods based on different mesh sizes to exclude soil organisms from litterbags, and not chemicals (e.g. naphthalene) ;
- at least two different mesh sizes had to be used for the litterbag experiment ;
- the experiments should be conducted *in situ* ;
- the experiments should not assess the effect of plant protection products on litter decomposition ;
- the publication had to be written in English, French or German (which excluded mostly Chinese and Portuguese papers).

Finally, 22 studies fit all of the previous criteria. These 22 studies were used to conduct our review and the data extracted from 17 of the 22 studies were used for the meta-analysis (see the list in supplementary material). In the case of studies that took interest in several land-uses, the following analyses on study parameters and results focus only on the data related to cropland.

5.2.2 Data extraction

For the 22 selected studies, we recorded the parameters related to geographical location (country), climate, soil type, standing crop and cropping system. In order to facilitate comparisons, climate types were denominated according to the five groups defined by the Köppen climate classification: tropical, dry, temperate, continental and polar; and soil types according to the FAO soil classes (World Reference Base for Soil Resources, 2014). When not given in the study, climate was deduced from the location of the study and by using regional climate maps, and soil types were collected in papers reporting experiments on the same sites.

We investigated the general design of the experiments: duration, number of sites and plots, number of replicate bags, number of sampling dates, sampling days, placement and depth of litterbags in soil. Information was also collected about the litterbags characteristics (bag size, mesh size, material, closure technique) and on the type of litter used in bags (litter quality, amount per bag, size of litter residues).

We collected data on litter decomposition. Twenty studies reported the decomposition as the percentage of remaining mass or initial mass loss in litterbags. Nine studies provided the decomposition rate, corresponding most of the time to the coefficient of the exponential model fitted on the data of remaining mass through time. If available, original data were extracted, otherwise they were obtained from graphics using the WebPlotDigitizer tool (Ankit Rohatgi, <https://automeris.io/WebPlotDigitizer>). If standard deviation, standard error or confidence interval were missing, we attributed the mean value of all standard deviations reported in studies selected for the same analysis. In addition, we summarized data on collected groups of soil organisms, sampling techniques and available measurements.

In order to gather decomposition data related to soil organisms of different size classes, we grouped mesh sizes used in the studies into three categories corresponding to the size classes of organisms that could enter bags. The fine mesh bags corresponded to bags with a mesh size lower than 0.1 mm, allowing access to litter to both microfauna (e.g. nematodes) and microflora (e.g. bacteria, fungi). The medium mesh bags had a mesh size comprised between 0.1 and 2 mm, allowing additional access to mesofauna which main representatives are microarthropods such as Collembola and Acari. Finally, the coarse mesh bags had a mesh size larger than 2 mm, thus enabling access to macrofauna organisms such as earthworms, carabids and other insects.

The role of the meso- and macrofauna in the decomposition of organic matter was determined from "paired observations" corresponding to the comparison between values of litterbag mass lost from two different mesh sizes (fine and medium, medium and coarse, or fine and coarse), studied under the same conditions. Each time point and each modality given in the studies provided individual paired observations with duration corresponding to the sampling

time. Thus, several paired observations were reported for each study. Hereafter, we will distinguish “study” (i.e. publication) and “paired observation” (i.e. result obtained in a study) with n the number of paired observations included in the analysis.

5.2.3 Data analyses

The mass loss rate (MLR) was calculated for each mesh group as $MLR = \%ML/t$, where $\%ML$ is the percent mass loss in litterbags at the time t (days). In addition, the fauna effect (FE) on litter mass loss was assessed by comparing the mass loss obtained in different bags such as $FE = \%ML_1 - \%ML_2$ where $\%ML_1$ and $\%ML_2$ are the percentage of mass loss in larger (coarse or medium) and smaller (medium or fine) mesh bags respectively (adapted from Seastedt, 1984). Statistical tests were conducted to assess if differences in mass loss rate were significantly affected by factors such as climate, soil, crop, agricultural systems and litter types, and to assess if overall macrofauna and mesofauna effects on decomposition were significantly different. For this purpose, the normality and homogeneity of variances were tested with a Shapiro-Wilk test ($\alpha > 0.05$) and a Bartlett test ($\alpha > 0.05$) using R package *stats*. As normality was not met, a Generalized Linear Mixed-effect Model (GLMM) with a gamma distribution, a fixed effect for the studied factor and a random effect for study level was applied, followed by Tukey HSD post-hoc tests. In order to obtain only positive values, data on the fauna effect were transformed by adding to each value the maximum fauna effect. Then, ANOVA with a χ^2 -test was performed to compare models with and without the studied factor. The mass loss or fauna effect were influenced by the studied factor if the Akaike Information Criterion (AIC) was significantly lower for the model with the factor than for the model without ($P < 0.1$).

The role of the meso- and macrofauna in litter decomposition was assessed by comparing mass loss in bags of different mesh size groups. The first set of analyses investigated the effect of macrofauna comparing coarse and medium mesh bags (based on the results of 12 studies containing 201 paired observations), the second set was on the effect of mesofauna comparing medium and fine mesh bags (10 studies, 126 paired observations), and the last set on the effect of combined macro- and mesofauna comparing coarse and fine mesh bags (7 studies, 75 paired observations). Paired observations with a duration lower than 14 days were not included in the statistical analyses, but were represented on the graphical representation of litter mass loss and fauna effect. Litterbags including several groups of organisms were considered as “treatment” and bags excluding one or several groups included in the treatment as “control” (coarse vs medium, medium vs fine and coarse vs fine respectively). The effect size (ES), or standardized mean difference between treatment and control, and the 95% confidence interval (CI) were computed for individual paired observations and overall results using the Hedges’g calculation (Hedges and

Olkin, 1985). A three-level random-effect model was applied, with random effects at the paired observation and at the study level. This allows to account for the lack of independence of paired observations from the same study, considering similar modalities but differing in sampling time. In addition, the heterogeneity between studies was calculated with the I^2 (Higgins and Thompson, 2002). Heterogeneity above zero reveals that the variability in the observed effect size is larger than one expected based on sampling variability. This may be explained by differences between studies according to one or several factors (e.g. climate, soil type), meaning that different subgroups of studies will have a significantly different mean effect size.

The role of different soil organisms in the decomposition of litter was compared for different categorical factors (climate, soil, crop, agricultural systems and litter type) and for continuous factors (depth of bags, duration of the experiment and size of litter pieces) considered as moderators to conduct a subgroup analysis. The effect of moderators was assessed with the Cochran Q-statistic and a χ^2 -test was performed to test its significance. A significant Q-statistic indicated that the moderator influenced the effect size ($P < 0.05$) and thus that part of the total heterogeneity could be explained by this difference. Except for climate, we only presented results for subgroups with more than 10 paired observations.

These analyses were performed using R software version 3.6.3 (R Development Core Team 2020) and the *lme4* (Bates et al., 2015), *multcomp* (Hothorn et al., 2008), *metafor* (Viechtbauer, 2010) and *dmetar* packages (Harrer et al., 2019).

5.3 Results

5.3.1 General characteristics of studies

The 22 selected studies were located all around the world, mostly across Europe (36%) but also in Asia (18%), South America (18%), North America (14%) and Africa (14%) (Table 5.1). In relation with their location, 54% of the studies occurred under temperate climate, and few studies under tropical (18%), continental (18%) and dry (9%) climates (Table 5.1). The soil type was highly variable. Several studies were conducted on Luvisol (24%) and Cambisol (14%) which are favorable to agriculture. The others occurred in nine different soil types (e.g. Ferralsol, Fluvisol, Lixisol, Kastanozem) (Table 5.1).

Most of the studies were conducted on annual crops under conventional agriculture (54%) and no-tillage agriculture (32%) (Figure 5.1a; Table 5.1). Only two studies were conducted under organic agriculture, one on minimum tillage and one on relay cropping. The most common crops at the time of the study were maize (32%), barley (23%), soybean (18%) and wheat (14%) (Figure 5.1b).

Collected studies had different objectives with some investigating the effect of climate (e.g. Peña-Peña and Irmeler, 2018; Yin et al., 2019), land-use (e.g. Tian et al., 1998; Yang et al., 2018) or litter type (e.g. Ouédraogo et al., 2004; Carlesso et al., 2019). They also focused on various agricultural practices, mainly tillage (e.g. House and Stinner, 1987; Domínguez et al., 2010), but also fertilization (Weil and Kroontje, 1979), conventional vs organic management (Domínguez et al., 2014; Yin et al., 2019) and genetically modified vs non-modified crops (Zwahlen et al., 2007). Finally, some authors also used litterbags to assess the decomposition activity of specific organisms (e.g. Jensen, 1985; Vreeken-Buijs and Brussaard, 1996).

Table 5. 1 Characteristics of studies selected for the meta-analysis. Time: duration of the study (days); n bag: number of replicates per modality; Size: size of bags (cm x cm); Depth: depth of bags placement in soil (cm); Fauna: studied groups of organisms.

N°	Publication	Location	Climate	Soil type	Cropping system	Time (days)	n bag	Size (cm)	Depth (cm)	Fauna	Litter
1	Broadbent & Tomlin, 1978	Canada	cont	Luvisol	C	112	8	11 x 11	5	-	maize
2	Carlesso et al., 2019	UK	temp	Cambisol	MT	180	6	5 x 6	5	-	ryegrass, wheat
3	Curry & Byrne, 1997	Ireland	temp	Gleysol	C	300	6	-	10	Lum	wheat
4	Domínguez et al., 2010	Argentina	temp	Kastanozem	NT	133	4	20 x 20	-	Ann, Ara, Colb, Ins, Lar, Lum, Myr	local, alfalfa
5	Domínguez et al., 2014	Argentina	temp	Kastanozem	C, O, NT	365	10	-	-	Colb, Lum, other	local, sorghum
6	Heisler, 1994	Germany	temp	Luvisol	C	270	4	10 x 10	10	Aca, Colb	wheat
7	House & Stinner, 1987	USA	temp	Acrisol	NT	120	9	10 x 10	0	Aca, Colb, other	clover, vetch, rye
8	Jensen, 1985	Denmark	temp	Luvisol	C	290	30	10 x 15	15	Lum, mesofauna	barley
9	Miura et al., 2008	Japan	cont	Andosol	C, NT	27	4-6	3.5 x 7	8; 18	Aca, Colb, Enc, mic, Nem, Pro	clover, wheat, sorghum
10	Ouédraogo et al., 2004	Burkina Faso	dry	Cambisol	-	90	4	15 x 18	3; 35	Ara, Colp, Der, Hym, Isop, Lum, Myr, Ort	maize, grass, dung
11	Peña-Peña & Irmeler, 2016	Brazil	trop	Ferralsol	NT	140	12	15 x 20; 20 x 20	0	-	soybean, maize
12	Peña-Peña & Irmeler, 2018	Brazil	trop	Ferralsol	C	140	12	15 x 20; 20 x 20	0	-	soybean, maize
13	Reddy et al., 1994	India	dry	Lixisol	C, NT	330	3	6 x 10	0	Aca, Colb, Isop, other	rice
14	Singh & Shekhar, 1989	India	temp	Luvisol	-	270	3	10 x 15	5; 12	-	wheat, maize
15	Tian et al., 1992	Nigeria	trop	Lixisol	-	98	4	30 x 30	0	-	tree leaves, maize, rice
16	Tian et al., 1998	Nigeria	trop	-	C, R	98	4	20 x 20	0	-	tree leaves
17	Valckx et al., 2011	Belgium	temp	Luvisol	NT	392	-	7 x 7	0	Lum	ryegrass, phacelia, mustard, rapeseed
18	Vreeken-Buijs & Brussaard, 1996	Netherlands	temp	Fluvisol	C	365	10	15 x 15	15; 25	Aca, Colb, Enc	wheat
19	Weil & Kroontje, 1979	USA	temp	Nitisol	C	70	4	10 x 15	8	Aca, Colb, mic	maize
20	Yang et al., 2018	China	temp	Fluvisol	-	365	4	15 x 20	-	Aca, Ara, Colb, Colp, Dip, Hym, Isod, Lep, Myr, Nem	tree leaves
21	Yin et al., 2019	Germany	cont	Chernozem	C, O	137	20	15 x 20	-	-	oat
22	Zwahlen et al., 2007	Switzerland	cont	Cambisol	C	240	7	10 x 10; 20; 10 20 x 20	20; 10	Aca, Ara, Colb, Enc, Isod, Lar, Lum, Myr, Nem, Pso	maize

cont: continental; temp: temperate; trop: tropical; C: conventional; MT: minimum tillage; NT: no-tillage; O: organic; R: relay cropping; Aca: Acari; Ann: Annelida; Ara: Araneae; Colp: Coleoptera; Colb: Collembola; Der: Dermaptera; Dip: Diptera; Enc: Enchytraeidae; Hym: Hymenoptera; Ins: Insecta; Isod: Isopoda; Isop: Isoptera; Lar: Larvae; Lep: Lepidoptera; Lum: Lumbricidae; mic: microflora; Myr: Myriapoda; Nem: Nematoda; Ort: Orthoptera; Pro: Protozoa; Pso: Psocoptera.

5.3.2 General design of litterbags experiments

Studies were mostly conducted on a single agricultural site (82%), while studies on several sites were scarce (18%, e.g. Domínguez et al., 2010; Peña-Peña and Irmeler, 2016) (Table 5.1). The number of plots ranged from one to 60 and plot size varied from 0.5 x 0.5 m to 120 x 30 m. However, studies with only one plot comprised 3 to 100 subplots within this plot, which we considered as plots.

Duration of experiments ranged from 27 to 392 days, being on average 206 days (Table 5.1). The number of sampling dates ranged from one to 10 (Figure 5.1c). Litterbags were mainly sampled between 5 and 120 days after their addition into soil, but in some studies they were sampled after more than 300 days (Figure 5.1d). Seven studies considered annual or seasonal dynamics and in this case, experiments lasted up to three years. Three types of sampling schemes were identified namely short studies with few sampling dates, long studies with few or many sampling dates.

The number of replicate bags per modality ranged from 3 to 30 (Table 5.1). Bags were placed on the surface (32%) or buried in soil between 3 and 35 cm (50%); however information on depth was missing in several studies (18%) (Table 5.1). Sometimes bags were placed on the surface and then buried at the time of tillage (e.g. Jensen, 1985; Domínguez et al., 2014).

5.3.3 Litterbag characteristics

Within the selected studies, 59% used litterbags with two mesh sizes (27% medium + coarse; 18% fine + medium; 14% fine + coarse), 32% with three (23% fine + medium + coarse; 4% fine + two medium; 4% coarse + two medium) and 9% with four mesh sizes (fine + coarse + two medium). The mesh size ranged from 0.003 to 10 mm (Figure 5.1e). Fine mesh ranged from 0.003 to 0.08 mm, medium mesh from 0.1 to 2 mm, and coarse mesh from 4 to 10 mm. The most common mesh sizes for each category were 0.02 mm (54%), 1 mm (29%) and 5 mm (31%).

The main material to construct litterbags was nylon or another synthetic polyester. Some studies however used stainless steel or brass in order to prevent bags from being decomposed by termites (tropical climate). The size of bags was mainly comprised between 10 x 10 cm and 20 x 20 cm (Table 5.1). The closure of bags was only discussed in seven publications. Bags were either sewn or glued.

Some authors improved the technique to limit litter losses in soil, after sampling and during transportation. For instance, Yang et al. (2018) sewed 0.01 mm mesh patches to the bottom of their litterbags. Jensen (1985) and Carlesso et al. (2019) used transportation bags or boxes, but they showed that mass loss during transportation was not significant in regards of the total mass

loss. Tian et al. (1992, 1998) placed pieces of wood inside litterbags to avoid litter compression. Finally, to rewet the litter and standardize the initial microflora in all litterbags, Vreeken-Buijs and Brussaard (1996) soaked all the litterbags in diluted filtered soil extract one night prior their incorporation into soil.

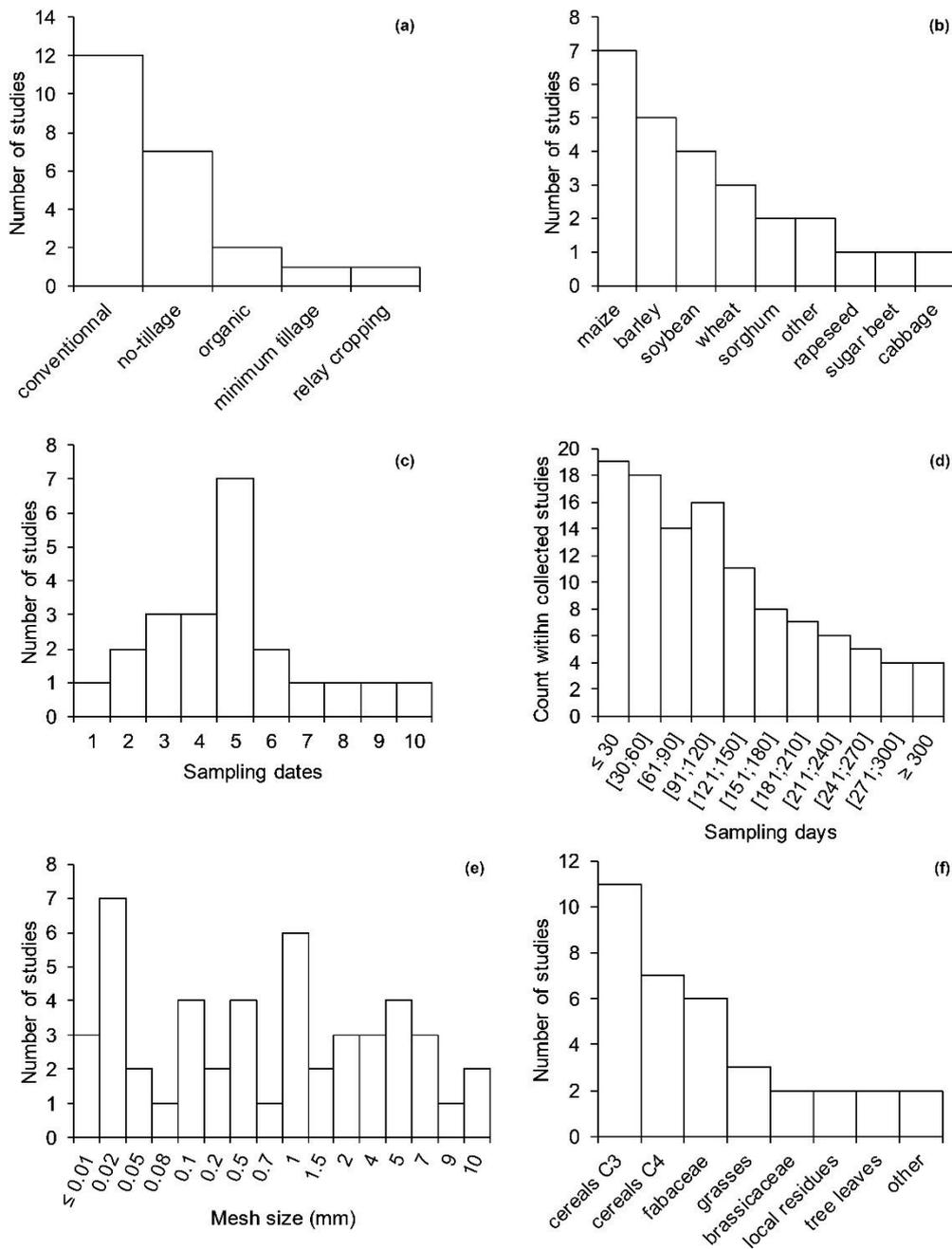


Figure 5. 1 Characteristics of litterbag experiments within selected studies (n = 22). (a) Cropping system. (b) Crop type. (c) Number of sampling dates. (d) Range of days within which sampling were conducted. (e) Size of mesh of litterbags. (f) Type of organic matter enclosed in litterbags.

5.3.4 Litter characteristics

Litter consisted of straw or leaves (Table 5.1). The most commonly used materials were C3 cereals (50%) such as wheat (27%) or rice (9%), C4 cereals (32%) such as maize (23%) or sorghum (9%), Fabaceae (27%) including crops (clover, vetch, alfalfa) and shrubs leaves (*Gliricidia sepium* (Jacq.) Steud., *Leucaena leucocephala* (Lam.) de Wit, *Senna siamea* (Lam.) H.S.Irwin & Barneby), and grasses (14%) (Figure 5.1f). Tree leaves (poplar, *Acioa barteri* (Hook.fil. ex Oliv.) Engl.), Brassicaceae (rapeseed, mustard) and manure were also used.

The C:N ratio of the litter, specified in six studies, ranged between 8 and 153. The C:N ratio was often the highest for C4 cereals (13 to 72), and remains high for C3 cereals (12 to 77). Comparatively, Fabaceae presented lower C:N ratios (9 to 57). However, C:N ratio was highly variable between studies for the same litter.

The amount of litter per bag ranged from 0.5 to 100 g, depending on the size of bags and the litter used. In some works, the size of residues was standardized by cutting litter into pieces of 1 to 10 cm length. Prior to introduction in bags, litter was often air or oven-dried between 20 °C (Valckx et al., 2011) and 105 °C (Carlesso et al., 2019).

5.3.5 Litter decomposition

The litterbags, once collected, were either brushed or rinsed under water to remove soil particles, roots, plant debris other than litter, and sometimes fauna. The bags were then dried between 20 and 105 °C, and weighed. In some cases, the litter was removed from the bags before being weighed. In nine studies, litter was burnt between 500 and 800 °C in order to measure ash free dry weight. Some studies performed additional analyses (e.g. C, N contents) to characterize the litter after biodegradation.

Enclosed litter lost more than half of its initial mass after a year or less in 57%, 46% and 23% of the coarse (n = 199 observations), medium (n = 254) and fine (n = 128) litterbags respectively (Figure 5.2). Mass loss rate was significantly influenced by climate in medium mesh bags only, and by soil and crop type in all mesh bags (Table S5.1). Agricultural systems did not significantly influence mass loss rate (Table S5.1). In medium mesh bags, mass loss was faster under tropical and continental climates than under dry (P < 0.001) and temperate climates (P < 0.05), and faster under temperate than under dry climate (P < 0.001) (Figure 5.2a-c). In addition, mass loss was faster in Acrisol than in Luvisol for all mesh bags (coarse and fine : P < 0.001, medium: P < 0.05), in Cambisol than in Luvisol in coarse (P < 0.001) and fine mesh bags (P < 0.001), and in Lixisol than in Luvisol in coarse (P < 0.001) and medium mesh bags (P < 0.01). Furthermore, mass loss

was faster in fields cropped with C4 cereals than with C3 cereals for medium ($P < 0.05$) and fine mesh bags ($P < 0.001$).

Litter decomposition was also significantly dependent on litter type, regardless of the mesh size (Figure 5.2d-e, Table S5.1). The degradation of C3 cereals was significantly slower than that of C4 cereals in medium ($P < 0.05$) and fine bags ($P < 0.001$), and slower than that of Fabaceae in all bags (coarse and medium: $P < 0.05$, fine: $P < 0.001$). More precisely, wheat decomposed significantly faster than maize in all bags (coarse, medium, fine: $P < 0.01$). It is noteworthy that C3 cereals, except for rice, were always used under temperate climate. In contrast, C4 cereals, especially maize, were used under all climate types.

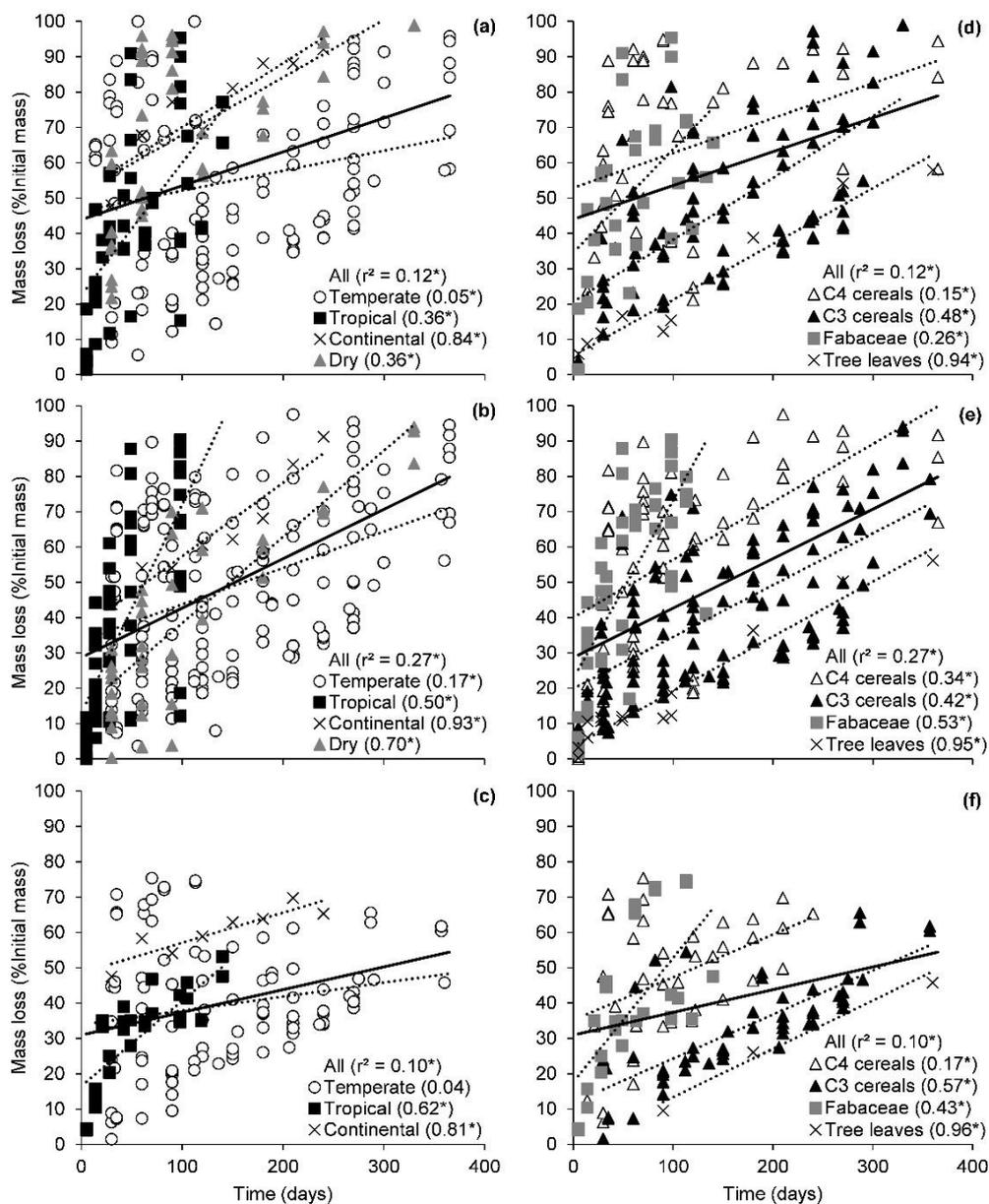


Figure 5. 2 Mass loss of organic matter in litterbags for different climates (a, b, c) and different litter qualities (d, e, f) in (a, d) coarse mesh ($n = 204$), (b, e) medium mesh ($n = 266$), and (c, f) fine mesh ($n = 130$) litterbags. Linear regressions were drawn for all data (continuous lines) and for different climates or litters (dotted lines). Coefficient of determination (r^2) are specified in brackets in the legend. Significant correlation are indicated by * ($P < 0.05$).

5.3.6 Effect of soil organisms on litter decomposition

In parallel to monitoring litter decomposition, the fauna was in some cases extracted from the soil (27%), from the litterbags (27%) or both (9%), using MacFayden or Berlese extractors for mesofauna (50%) and hand sorting for macrofauna (45%). Microorganisms were studied in 18% of the studies. Seven studies considered two size classes and only one the three size classes (Miura et al., 2008). The most studied groups of soil fauna were Collembola (45%), Acari (36%), Lumbricidae (32%), Araneae (18%), Enchytraeidae (14%) and Nematoda (14%) (Table 5.1). However, some studies also took interest at Coleoptera, Isoptera, Myriapoda (Chilopoda, Diplopoda, Symphyla, Pauropoda), Crustacea (Isopoda), Annelida, Dermaptera, Diptera, Hymenoptera (Fomicidae), Lepidoptera, Orthoptera, Psocoptera, and larvae (Coleoptera, Diptera) (Table 5.1). Most of the studies assessed organism's density or biomass, while diversity (richness, diversity indexes) was only discussed in five studies.

Microbial activity was characterized *via* respiration (CO₂ measurements) or substrate-induced respiration. Only one study focused on functional groups, gathering together microarthropods according to their feeding habits (Vreeken-Buijs and Brussaard, 1996). Finally, six studies assessed the "fauna effect" as the difference of mass loss between bags including and excluding soil fauna.

5.3.6.1 Role of macrofauna

Soil macrofauna had a significant positive effect on the decomposition of organic matter in litterbags with a mean fauna effect of 9.4% (Figure 5.3a, d; range = [-20.3%, 83.5%]) and a mean effect size of 0.66 (Figures 5.4a and S1; 95% CI = [0.37; 0.96], $P < 0.0001$, $n = 201$ paired observations). A moderate heterogeneity between studies was observed ($I^2 = 72.3\%$). A negative mean fauna effect occurred in 22.2% of the paired observations (Figure 5.3a, d).

The effect of macrofauna on litter decomposition was dependent on the climate ($P < 0.0001$), soil ($P < 0.0001$), crop types ($P < 0.0001$) and agricultural systems ($P = 0.0003$) (Figure 5.4b, Table S5.2). Macrofauna effect on decomposition was the highest under dry (ES = 3.12; $n = 36$ paired observations) and continental (1.40; $n = 8$ from only 1 study) climates (Figure 5.4b), being especially high during the early stages of decomposition and then decreasing with time (Figure 5.3a). In comparison, it was lower under tropical (0.28; $n = 40$ from 1 study) and temperate (0.23; $n = 117$) climates (Figure 5.4b), for which it remained quite steady over time (Figure 5.3a). Furthermore, the macrofauna played a particularly important role for the litter degradation in Cambisols (4.19; $n = 26$), and had a lower but significant effect in Nitisols (0.70; $n = 12$ from 1 study), Luvisols (0.53; $n = 25$), Gleysols (0.43; $n = 30$ from 1 study) and Lixisols (0.31; $n = 58$)

(Figure 5.4b). It was not significant in Kastanozem soils (0.14; $n = 22$) (Figure 5.4b). Conversely, the effect size was significantly negative in Acrisols (-0.26; $n = 24$ from 1 study). The effect of macrofauna also varied with the standing crop, with a maximum effect size observed in sorghum fields (3.12; $n = 36$) followed by wheat (0.82; $n = 10$), barley (0.49; $n = 53$) and soybean fields (0.49; $n = 10$). It was not significant under maize fields (0.10; $n = 82$) (Figure 5.4b). However, it was difficult to discriminate the effects of dry climate and sorghum cultivation as they co-occurred in the same studies (Reddy et al., 1994; Ouédraogo et al., 2004). Finally, macrofauna demonstrated a significant positive effect on decomposition under conventional systems (0.53; $n = 77$), and a rather low and not significant effect under no-tillage systems (-0.04; $n = 50$) (Figure 5.4b).

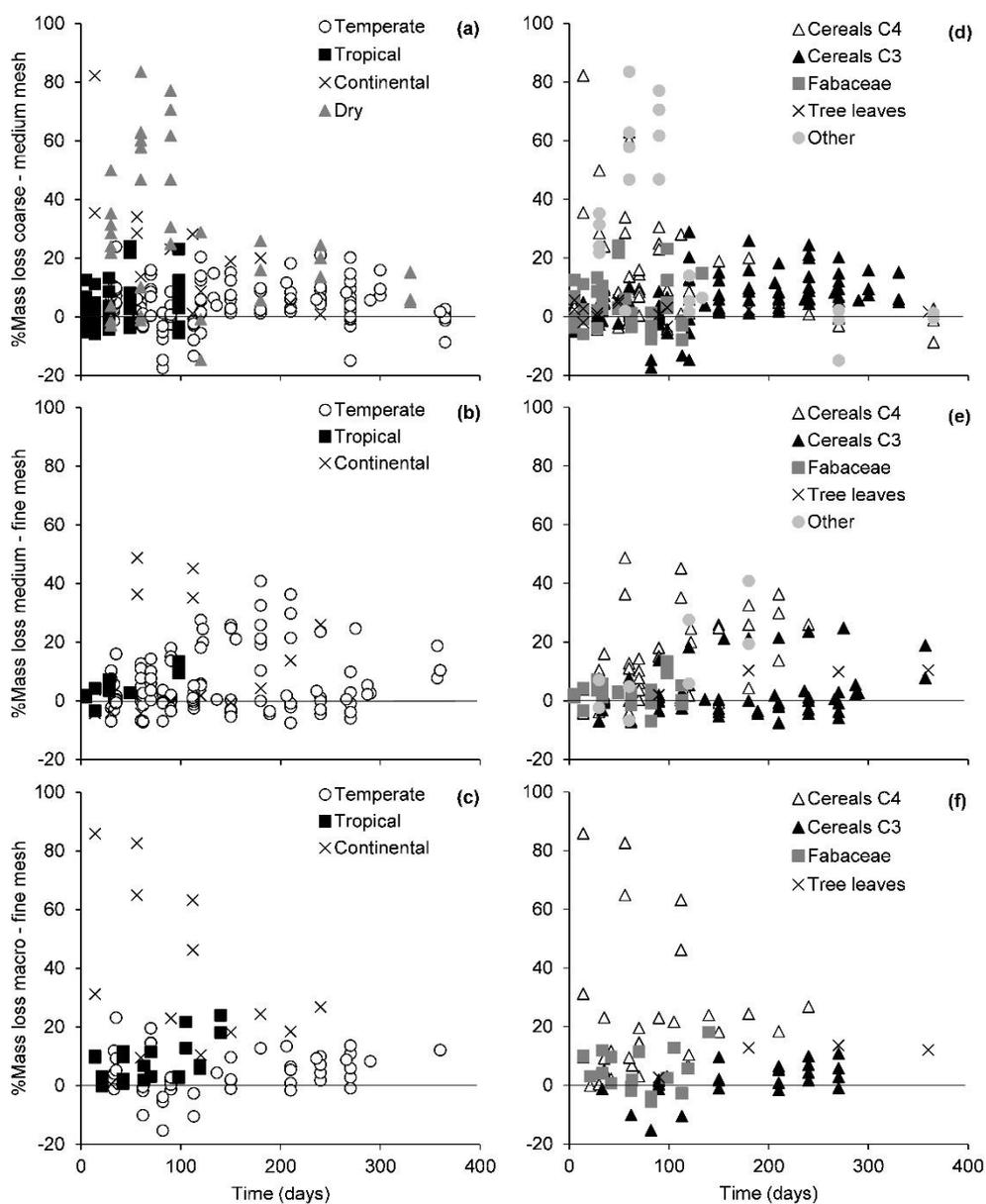


Figure 5. 3 Temporal evolution of the role of soil fauna on litter decomposition in relation to climate (a, b, c) and litter types (d, e, f) with the effect of (a, d) macrofauna ($n = 217$), (b, e) mesofauna ($n = 134$), and (c, f) both macrofauna and mesofauna ($n = 81$). Lines indicate the limit between positive and negative fauna effect.

The type of litter did not significantly influenced the effect of macrofauna ($P = 0.11$) (Figure 5.4b, Table S5.2). However, the macrofauna was observed to play a very important role in the degradation of C4 residues such as maize (1.41; $n = 34$) (Figure 5.4b), especially in the early stages of degradation (Figure 5.3d). Its role was less important and later for C3 residues such as wheat (0.42; $n = 50$) and rice (0.42; $n = 26$) (Figure 5.4b). It was not significant for local residues (0.16; $n = 11$) (Figure 5.4b). The macrofauna effect fluctuated for Fabaceae and tree leaves for which it was sometimes positive or negative (Figure 5.3d).

The depth of bags had a positive significant influence on the effect of macrofauna ($P < 0.0001$), but not the duration of the incubation in field ($P = 0.14$) or the size of the litter pieces ($P = 0.87$) (Table S5.3).

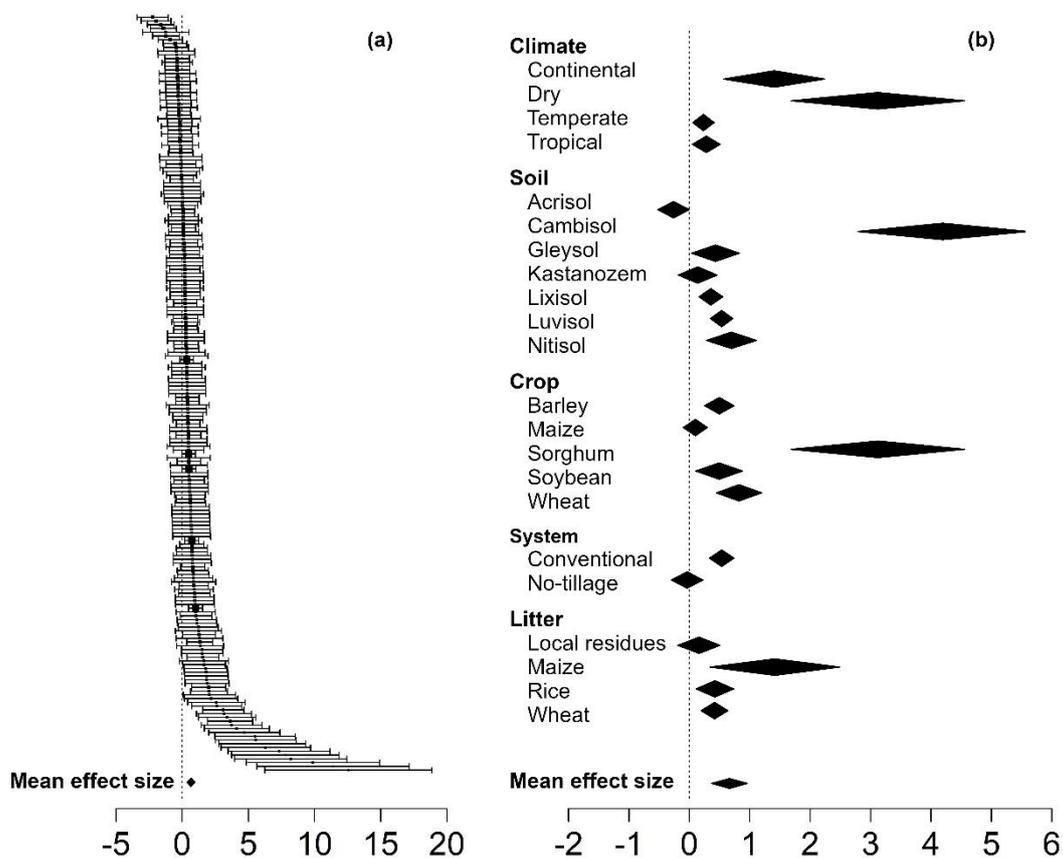


Figure 5. 4 Effect size of macrofauna on litter decomposition. Represents the effect size of all the paired observations comparing coarse versus medium mesh litterbags (incl. /excl. macrofauna) with (a) the effect sizes of all paired observations ($n = 201$) and (b) the effect sizes for each climate, soil, crop, agricultural systems and litter subgroups. "Mean effect size" represents the overall effect size of all paired observations. Effect size of each paired observation and the associated 95% confidence interval are represented by (a) black squares and bars or (b) center and length of diamonds.

5.3.6.2 Role of mesofauna

Soil mesofauna had a significant positive influence on the decomposition of organic matter in litterbags with a mean fauna effect of 6.6% (Figure 5.3b, e; range = [-7.5%, 48.7%]) and a mean effect size of 0.31 (Figures 5.5a and S5.2; 95% CI = [0.11; 0.52], $P = 0.003$, $n = 126$ paired observations). The heterogeneity was low ($I^2 = 40.2\%$). A negative mean fauna effect appeared in 29.5% of the paired observations (Figure 5.3b, e).

The effect of mesofauna was significantly influenced by the crop types ($P = 0.003$), while no significant difference was found between different climates ($P = 0.99$), soils ($P = 0.57$) or agricultural systems ($P = 0.06$) (Figure 5.5b, Table S5.2). Mesofauna effect on decomposition was significantly positive under temperate climate only (ES = 0.31, $n = 110$ paired observations), but not under continental (0.49, $n = 8$) and tropical climates (0.36, $n = 8$) for which it increased with time (Figure 5.3b). The lack of difference for the climate subgroup could be explained as almost all the experiments were conducted under temperate climate ($n = 110$). Moreover, mesofauna had a non-significant positive effect on decomposition in Fluvisols (0.67; $n = 14$), Cambisols (0.59; $n = 20$), Luvisols (0.42; $n = 48$) and Nitisols (0.33, $n = 12$), and a non-significant negative effect in Acrisols (-0.01; $n = 24$ from 1 study) (Figure 5.5b). In addition, mesofauna played an important role on the litter decomposition in wheat fields (1.35; $n = 14$) compared to rapeseed (0.64; $n = 12$ from 1 study), maize (0.18; $n = 45$), barley (0.09; $n = 33$) and sugar beet fields (0.05; $n = 10$ from 1 study) for which its effect was not significant (Figure 5.5b). Finally, a significantly positive mesofauna effect on decomposition was observed under conventional systems only (0.14; $n = 49$).

Mesofauna effect on decomposition was also dependent on the litter type ($P = 0.03$) (Figure 5.5b, Table S5.2). The mesofauna particularly increased the decomposition of C4 cereals such as maize (0.65; $n = 34$) (Figure 5b), especially at the early stage of decomposition (Figure 5.3e). Comparatively, it did not significantly increase the degradation of C3 cereals such as wheat (0.09; $n = 43$) (Figure 5.5b), with low or negative effect, only increasing at the later stage of the decomposition (Figure 5.3e). Finally, the mesofauna effect tended to increase with time for tree leaves (Figure 5.3e) and fluctuated for Fabaceae (Figure 5.5b).

The duration of the experiment significantly influenced the effect of mesofauna on decomposition ($P = 0.002$), but not the depth of litterbags ($P = 0.98$) or the size of litter pieces ($P = 0.71$) (Table S5.3).

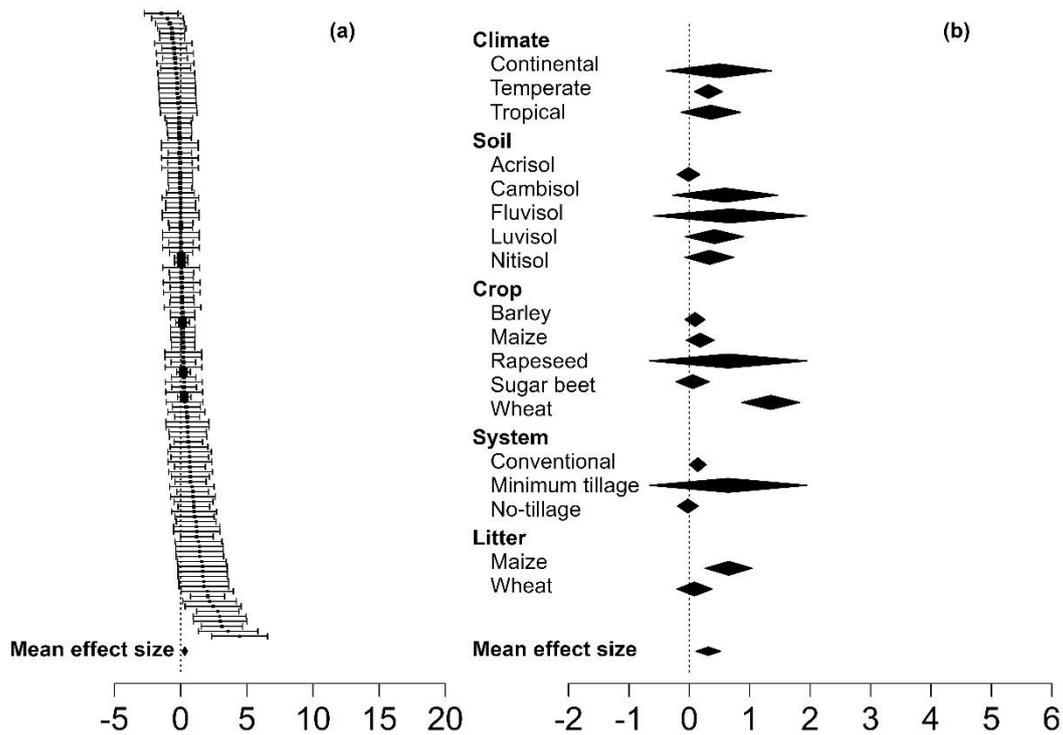


Figure 5.5 Effect size of mesofauna on litter decomposition. Represents the effect size of all the paired observations comparing medium versus fine mesh litterbags (incl. /excl. mesofauna) with (a) the effect sizes of all paired observations ($n = 126$) and (b) the effect sizes for each climate, soil, crop, agricultural systems and litter subgroups. "Mean effect size" represents the overall effect size of all paired observations. Effect size of each paired observation and the associated 95% confidence interval are represented by (a) black squares and bars or (b) center and length of diamonds.

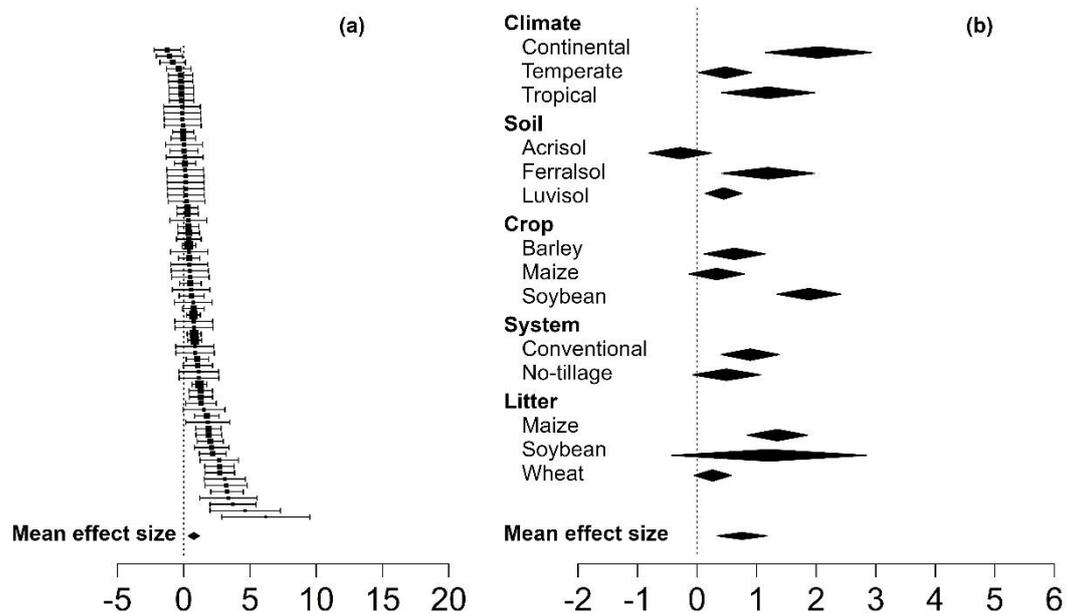


Figure 5.6 Effect size of total fauna on litter decomposition. Represents the effect size of all the paired observations comparing coarse versus fine mesh litterbags (incl. /excl. macro- and mesofauna) with (a) the effect sizes of all paired observations ($n = 75$) and (b) the effect sizes for each climate, soil, crop, agricultural systems and litter subgroups. "Mean effect size" represents the overall effect size of all paired observations. Effect size of each paired observation and the associated 95% confidence interval are represented by (a) black squares and bars or (b) center and length of diamonds.

5.3.6.3 Combined impact of both macro- and mesofauna

Soil macro- and mesofauna together contributed positively and significantly to the decomposition of litter compared to microorganisms only. The mean fauna effect of combined macro- and mesofauna on litter decomposition was 10.8% (Figure 5.3c, f; range = [-15.3%, 85.9%]) and the mean effect size was 0.75 (Figures 5.6a and S5.3; 95% CI = [0.34; 1.17], $P = 0.0004$, $n = 75$ paired observations) (Figure 5.6a). A moderate heterogeneity was observed ($I^2 = 73.75\%$). The mean fauna effect was negative in 18.5% of the paired observations (Figure 5.3c, f). The cumulative effect was lower than the effect obtained by the addition of the macrofauna and the mesofauna effects that reached 16.0%. In addition, it should be noted that the individual fauna effects of macrofauna and mesofauna were not significantly different ($P = 0.36$).

The fauna effect on decomposition was dependent on soil ($P = 0.002$) and crop type ($P = 0.01$), but not on climate ($P = 0.10$) or agricultural system ($P = 0.32$) (Figure 5.6b, Table S5.2). Soil fauna played a very important role in the litter decomposition under continental climate (ES = 2.04; $n = 8$ paired observations from 1 study) (Figure 5.6b), especially at the early stages of decomposition (Figure 5.3c). It was also important under tropical climate (1.19; $n = 20$ from 1 study) (Figure 5.6b), for which it increased with time (Figure 5.3c). It tends to be lower under temperate climate (0.47; $n = 47$) (Figure 5.6b) and seemed quite steady over time (Figure 5.3c). In addition, soil fauna provided a significant increase in litter decomposition in Ferralsols (1.19; $n = 20$ from 1 study), and to a lesser extent in Luvisols (0.45; $n = 25$) (Figure 5.6b). Contrastingly, soil fauna effect was negative but non-significant in Acrisols (-0.29; $n = 12$ from 1 study) (Figure 5.6b). Nevertheless, it was not possible to discriminate the effects of tropical climate and Ferralsol soil type as they co-occurred in one study (Peña-Peña and Irmeler, 2016). Furthermore, soil fauna positively influenced litter decomposition in soybean (1.88; $n = 10$ from 1 study) and barley fields (0.63; $n = 33$), and not significantly in maize fields (0.33; $n = 28$) (Figure 5.6b).

The implication of soil fauna also depended on the litter type ($P = 0.03$) (Figure 5.6b, Table S5.2). Following the trends separately observed for macro- and mesofauna, soil fauna was highly involved in the degradation of C4 cereals such as maize (1.35; $n = 24$) (Figure 5.6b), particularly at the early stage of decomposition (Figure 5.3f). In addition, it demonstrated a positive but non-significant effect on Fabaceae such as soybean (1.21; $n = 10$) (Figure 5.6b), slightly increasing with time (Figure 5.3f). This effect was lower and not significant for C3 crops such as wheat (0.26; $n = 20$ from 1 study) (Figure 5.6b), for which it was negative at the early stage of decomposition and increased at the later stages (Figure 5.3f). Finally, fauna effect tended to increase with time for the decomposition of tree leaves (Figure 5.3f).

The duration of the experiment ($P = 0.01$) had a significant positive influence on fauna effect, such as the size of litter pieces ($P < 0.0001$) which indicates that large litter pieces required more

intervention of fauna than small litter pieces. No significant relations were found with the depth of litterbags ($P = 0.64$) (Table S5.3).

5.4 Discussion

5.4.1 Role of soil organisms in litter decomposition

The role of the soil organisms on litter decomposition in cropping systems was confirmed as our meta-analysis demonstrated a significant positive effect of macrofauna (FE = 9.4%, ES = 0.66), mesofauna (FE = 6.6%, ES = 0.31) and combined macro- and mesofauna (FE = 10.8%, ES = 0.75). These results are consistent with previous reviews and meta-analyses assessing the role of macrofauna (Frouz et al., 2015), mesofauna (Seastedt, 1984; Kampichler and Bruckner, 2009) and total soil fauna (Zhang et al., 2015) on litter decomposition.

Macrofauna and mesofauna effects on decomposition were not significantly different in annually cropped soils. Similarly, in forest soils the overall effects of macrofauna and mesofauna were not reported to differ, but to vary in different ecosystems (González and Lodge, 2017; Krishna and Mohan, 2017). The balance between macrofauna and mesofauna effects could be due to the distinct roles of the main macrofauna and mesofauna organisms and to the variation in taxa within both groups.

Macrofauna organisms are highly efficient for the burrowing, breakdown and digestion of litter, thus displacing a large amount of litter. After digestion most of the organic matter is returned to the soil in the form of feces (Frouz, 2018). However, the assimilation efficiency varies greatly among the different groups of macrofauna (Frouz, 2018). In the collected studies, earthworms were very often the main macro-decomposers. Earthworms are essential for the mixing of litter in soils and for its transformation through comminution (Barrios, 2007). They consume organic matter together with soil, which results in the creation of microaggregates in their feces with a reduced decomposition rate of organic matter compared to the initial litter (Frouz, 2018). Under dry climates, the high macrofauna effect was mostly attributed to termites that are reported to be one of the main actors of litter decomposition in arid areas (Martínez-Yrizar et al., 2007). Termites have a high assimilation efficiency allowing them to convert a great proportion of the ingested litter into their own biomass (Culliney, 2013), thus releasing less organic matter directly after the digestion of litter.

Mesofauna effect on decomposition is, on the other hand, mostly indirect and attributable to microarthropods such as Collembola and Acari (Culliney, 2013). They take part in decomposition through the digestion and the breakdown of litter, but their effect on decomposition is mostly due to the regulation of microbial populations by grazing (Seastedt, 1984). Indeed, oribatid mites and

Collembola have mouthparts adapted to the fragmentation of organic matter that enable them to feed on the adhering microorganisms (Seastedt, 1984). In addition, predatory microarthropods feeding on bacterial and fungal nematodes can also protect microbial populations and stimulate litter decomposition (Santos et al., 1981). The regulation of microorganisms by microarthropods was suggested to ensure continuous and regulated supply of nutrients to plants and thus to prevent nutrient loss (Culliney, 2013).

Despite the observed mean positive effect, several studies related a negative effect of soil fauna on decomposition (e.g. House and Stinner, 1987; Heisler, 1994). Those studies were not sharing specific characteristics. A main driver of the effects of macrofauna and mesofauna could be the balance between predatory and prey organisms within these two groups. Previous studies demonstrated that in sites dominated by predaceous macrofauna the litter mass loss in coarse bags decreased due to the predation on mesofauna prey (Frouz et al., 2015). In addition, the positive effect of mesofauna on decomposition may be counterbalanced by an over-grazing of microorganisms by microarthropods (Beare et al., 1992; Heisler, 1994), which could conduct to null or negative total observed effect on decomposition.

Furthermore, the effects of macrofauna and mesofauna were not additive as the mean fauna effect, combining macrofauna and mesofauna, was lower than the addition of the two effects. The non-additive aspect of the fauna effects on decomposition could be explained by the existence of facilitative (e.g. resource use complementarity) and inhibitory (e.g. competition, predation) interactions between soil organisms (Heemsbergen et al., 2004). These interactions are assumed to explain why the combined effect of soil organisms on litter decomposition cannot be predicted with the simple sum of their individual effects (Coulis et al., 2015).

Litter decomposition was increasingly faster in fine, medium and coarse mesh litterbags respectively, which may indicate an effect of the inclusion of a larger group of soil fauna. However, the protocols set up in the selected studies allow studying the role of macrofauna and mesofauna in litter decomposition, but they do not allow discussing the role of microorganisms, which are present in all litterbags. Still, more than aiming to estimate the individual effect of soil microorganisms, the focus of future studies should be set on the interactions between soil organisms and the parameters that promote microbial activity.

5.4.2 Factors explaining the variability of the effect of soil fauna

Litter mass loss and fauna effect on decomposition were both influenced by several factors. This implies that the studied factors had direct effects on litter decomposition, but also indirect effects through their impact on soil organisms. In the following section, we will discuss the influence of climate, soil, agricultural systems, crop and litter type on the fauna effect on litter

decomposition. We aim here to illustrate the trends that are observed within the meta-analysis, even though more studies would be required to conclude on the impact of these factors. In addition, one should keep in mind the existence of various interactions among studied factors (e.g. climate and soil).

5.4.2.1 Climate

Overall, macrofauna, mesofauna and total fauna effects tend to be higher under dry and continental climates, and were lower under temperate climate (Figures 5.4-6). Climate and more specifically temperatures and precipitations are important drivers of litter decomposition (Knacker et al., 2003; Zhang et al., 2015) by influencing the decomposition activity of soil organisms (García-Palacios et al., 2013; Zhang et al., 2015). Litter decomposition tends to be optimum for intermediate humidity and temperature; however, the optimum will depend on the local decomposer communities and on the presence of adapted species (Knacker et al., 2003; Peña-Peña and Irmler, 2016). For instance, Peña-Peña and Irmler (2016) found a much higher fauna effect on decomposition in cropland during the wet season than during the dry season. Contrastingly, Kampichler and Bruckner (2009) concluded to a significantly higher effect of microarthropods in arid areas than in wet tropics, all land-uses together. In a two-year experiment, Yin et al. (2019) also demonstrated that a slight increase in temperature and change in precipitation patterns was enough to decrease the effect of soil fauna on litter decomposition.

5.4.2.2 Soil type

The soil type influenced litter mass loss in bags of all sizes as well as the macrofauna (Figure 5.4) and the combined macro- and mesofauna effects on decomposition (Figure 5.6), but not the mesofauna effect (Figure 5.5). The observed positive fauna effect on decomposition in Luvisols and Cambisols may be explained as they are fertile soils highly adapted to agricultural use and that do not present any particular constraints for soil organisms. Furthermore, the high variability in the fauna effect in Cambisols is explained as Cambisols are considered as “non-differentiated” soils that have heterogeneous properties. As the most represented soil types, Luvisols and Cambisols occurred in combination with different climates but it was not possible to compare their effect under similar climate. Moreover, the literature on the influence of different soil types on soil organism’s communities in agricultural soils is scarce (Girvan et al., 2003; Ivask et al., 2008; Khan and Joergensen, 2012; Zaller et al., 2014). In addition, the few existing studies did not look at the effect of soil types on the functions performed by soil organisms. Thus, further studies are required to unveil the mechanisms causing soil types to influence the role of different soil

organisms in decomposition. Furthermore, soil characteristics, rather than soil types only, are required to understand the impact of different soils on organism's decomposition activity.

5.4.2.3 Crop

Crops cultivated on the field at the time of litterbag experiments influenced the litter mass loss in all bags and the fauna effects on decomposition (Figures 5.4-6), with a significant positive effect in fields with wheat and barley (except for barley with mesofauna), and lower and non-significant effect in fields with maize. Few studies have been conducted on the effect of crop type on soil biodiversity. They demonstrated crop type influence on soil organism community composition, abundance and diversity (Eyre et al., 2012; Lüscher et al., 2014; Crotty et al., 2015; Detheridge et al., 2016; Villenave et al., 2018). Crop types were notably found to influence the abundance of predators in fields such as spiders (Lüscher et al., 2014) and of bacterivorous nematodes (Villenave et al., 2018). Crops present specificities such as plant structure, roots distribution or root exudates that may favor different organisms. In addition, crops are associated with different practices such as the frequent use of irrigation for maize crops or the relatively low need for chemicals input in wheat fields. Besides, crops previously cultivated on the field were found to influence soil organisms even after the establishment of a new crop (Crotty et al., 2016). The numerous differences between crop type and their management make difficult to explain the specific effect of crops on the activity of decomposers. Further studies are required on this point.

5.4.2.4 Litter

The nature of organic matter added in litterbags greatly influenced the mass loss rate in litterbags of all mesh sizes. It also influenced the effects of mesofauna (Figure 5.5) and combined macro- and mesofauna (Figure 5.6). Macro- and mesofauna considerably increased the degradation of C4 cereals litter, especially at the early stage of decomposition. Contrastingly, they demonstrated low or no influence on the decomposition of C3 crops such as wheat. Their influence on Fabaceae and other crops was more variable and fluctuated with time. The limited availability of data on litter composition in the selected papers prevented us to observe if there was a relation between litter characteristics (e.g. percentages of soluble and more recalcitrant C, phosphorus, C:N ratio) and decomposition. In our study, we observed a higher fauna effect for maize than for wheat litter regardless of the climate and the soil type. It was surprising as maize tends to have a high C:N ratio in the collected studies, probably because of its high fiber and lignin content (Sereda et al., 2015). Whereas in previous studies, litters with low C:N ratio in cropping systems were

sometimes found to decompose faster (Vazquez et al., 2003) and to present higher fauna effect than litter with high C:N ratio (Zhang et al., 2015; Peña-Peña and Irmeler, 2018).

Another potential explanation for the higher fauna effect on maize litter could be the home-field advantage effect. Indeed, there is increasing evidence that litter decomposes faster beneath the plant from which it was derived, the so-called home-field advantage effect, because of the adaptation of the local soil community to the standing plant species, either by adapted activities, changes in community composition or a combination of several processes (Milcu and Manning, 2011). The home-field advantage effect increased litter decomposition rate by 7.5% for local litter according to Veen et al. (2015). In addition, the home-field advantage effect was demonstrated to be higher for more recalcitrant litter as specialized decomposer communities are required to degrade it rapidly (Milcu and Manning, 2011). In our meta-analysis, half of the experiments using maize litter were conducted under maize fields, thus with a potential home-field advantage effect, while only few experiments using wheat litter were conducted under wheat fields.

5.4.2.5 Agricultural practices

Agricultural systems significantly influenced macrofauna effect on decomposition (Figure 5.4) but not litter mass loss in bags or other fauna effects in our study (Figures 5.5 and 5.6). Macrofauna effect on decomposition was significantly higher in conventional than in no-tillage systems. Conversely, previous litterbag studies found no-tillage systems to exhibit higher decomposition than conventionally tilled systems (Reddy et al., 1994; Miura et al., 2008; Faust et al., 2019), but the contrary was also found (Domínguez et al., 2014). Agrosystems under conventional tillage and no-tillage management experience vastly different disturbance regimes, which affect the soil food web (Wardle, 1995). Even if the difference between systems was not significant in our study, the effect of macrofauna, mesofauna and their combined effect tend to increase litter decomposition in conventional systems but not in no-tillage systems. This is surprising as macrofauna and mesofauna densities are reported to be higher under low tillage systems, notably for macroarthropods (Reddy et al., 1994), earthworms (Parmelee et al., 1990; Miura et al., 2008) and microarthropods (Brennan et al., 2006; Dubie et al., 2011). In addition, larger organisms tend to be more sensitive to tillage than smaller organisms (Wardle, 1995; Kladvko, 2001). Tillage can indeed affect soil organisms by direct impacts, such as physical damages or trapping in soil pores (Kladvko, 2001), or indirect impacts such as changes in soil moisture and in organic matter distribution, and destruction of microhabitats for decomposers (Reddy et al., 1994; Tsiafouli et al., 2015). The lack of available information prevented us from conducting further statistical analyses on the effects of agricultural practices. Yet, different

practices unevenly affect soil organisms, conducting to changes in community composition, which can in turn strongly affect litter decomposition (Bradford et al., 2002).

5.4.2.6 Temporal evolution

The effect of macrofauna, mesofauna and both together varied with time, but with patterns depending on the context. For instance, under dry and continental climates and for C4 cereals litter fauna effects were higher at the early stage of decomposition and decreased with time. In contrast, they increased slowly with time under temperate and tropical climates and for C3 cereals litter.

The variation in the effect of soil fauna may be related to the succession of organisms in litterbags. Soil organisms successively colonize litter according to their growth and migration rate (Georgieva et al., 2005). Several studies found bacteria or fungi to successively dominate the process of crop residues decomposition, with the presence of associated predators controlling their activity (Vreeken-Buijs and Brussaard, 1996; Georgieva et al., 2005; Jacobs et al., 2011). In addition, the highest mesofauna effect on crop residues was demonstrated at the early (Beare et al., 1992) and intermediate (Lekha et al., 1989) stages of litter decomposition. The intervention of macrofauna organisms could occur depending on their feeding habitats, respectively in early stage of decomposition if they feed directly on litter and later for predators that feed on decomposer organisms.

5.4.3 General remarks on the meta-analysis

Our analysis sometimes faced confounding effects between the different factors. For instance, studied crops were different under different climates, with a predominance of C3 crops under temperate climate and C4 crops under dry climate. In addition, some factors occurred only once and in the same study, such as climate and soil type for instance, due to the lack of studies under various climates (e.g. continental, tropical, dry) and soil types (e.g. Ferralsol). Furthermore, the effect of agricultural systems on decomposition was not clear in our analysis. However, previous case studies found alternatively management practices and soil types to have more influence on soil microarthropods and microorganisms (Fromm et al., 1993; Reeleder et al., 2006). Resolving the share of the influence of agricultural practices and soil type in decomposition could lead to reinterpret the effect of soils on decomposition and to attribute the observed effects to agricultural management rather than to soil type only. Future litterbags studies may help to characterize soil fauna and litter decomposition for several agricultural practices under a diversity of pedoclimatic contexts.

In addition, the results of our meta-analysis show a high publication bias (Figure S5.4; Rothstein et al., 2006), with a prevalence of broad studies with significant results over small and non-significant ones. This bias could lead to over- or underestimate the comparative effect of organism's size classes. Thus, we would like to encourage authors and reviewers to allow for the publications of litterbag studies in agricultural areas, even if the results do not show significant differences.

5.4.4 Advantages and limitations of the litterbag method in cropped soils

Allowing for studies *in situ*, litterbags may provide a better estimate of the decomposition rate and fauna effect than microcosm experiments. This is especially true in cropping systems as they are submitted to various disturbances that are difficult to mimic in laboratory. In a previous review, Knacker et al. (2003) concluded that the litterbag method has more advantages than other approaches (i.e. minicontainer, cotton strip, detection of isotopes, bait-lamina) as it allows to apply numerous measurement endpoints to assess litter decomposition under field conditions (e.g. number of invertebrates, enzyme activity, chemical composition, mass loss of litter). In addition, litterbags constitute a relatively easy and cost-effective method to assess litter decomposition and soil organism activity in cropped soils. They require little space and no specific equipment, and allow for short and long-term experiments, depending on the agricultural practices in place, with numerous replicates and treatments. Furthermore, enclosed litter gives a relatively good estimate of the decomposition of crop residues buried or at the surface of agricultural soils (Ouédraogo et al., 2004).

More generally, several authors suggested that the enclosure of litter create artificial conditions that can prevent accessing the real decomposition rate (Anderson, 1973; Bradford et al., 2002; Prescott, 2005). They notably suggested that a “mesh size effect” could explain part of the differences observed between bags of different mesh sizes. Fine mesh litterbags were reported i) to be subjected to microclimate with an increased moisture responsible for an over-estimation of the results (Irmiler, 2000; Bradford et al., 2002), ii) to constitute a physical barrier that slow colonization of litter by microorganisms (Wise and Schaefer, 1994), and iii) to be colonized by a small fraction of soil mesofauna through egg deposition (House and Stinner, 1987; Vreeken-Buijs and Brussaard, 1996). In contrast, bags with a larger mesh size were found i) to allow organisms to pull litter from the bags even without degrading it (Bradford et al., 2002), ii) to increase the risk of litter loss during the handling of bags or leaching due to rainfall (Kampichler and Bruckner, 2009), and iii) to ease the admission of soil particles and roots within bags that washing or brushing after recollection do not completely removed, which increase the final weight of litter but also increase the surface of soil-litter contact and therefore the microbial decomposition

activity within bags (Broadbent and Tomlin, 1978; House and Stinner, 1987; Heisler, 1994). Nevertheless, the scientific community considers litterbags as a useful method for comparative studies and it is admitted that potential bias will be similar between experiments, allowing the comparison of data collected with bags of similar mesh size (Domínguez et al., 2014; Peña-Peña and Irmeler, 2016).

5.4.5 Adapting the litterbag protocol to agricultural areas

Existing studies on litterbag experiments occurring in cropping systems enabled to provide interesting results on the role of soil organisms in litter decomposition. However, forecasting future studies on soil biodiversity in an agricultural context, we recommend the development of a standard protocol adapted to the specificities of cropped soils. The standardization of the litterbag protocol is necessary in order to increase the number of studies in cropped soils and to ease the comparison of results. Previous authors already advocated for the need of a standard protocol for litterbag experiments and a methodology to assess litter decomposition (Knacker et al., 2003; Kampichler and Bruckner, 2009; Krishna and Mohan, 2017). However, to this day, we are not aware of the publication of a standardized protocol to assess the effect of soil organisms on litter decomposition in agricultural soils. There is no standard design and the litterbags used for current experiments are handmade with the available means. Guidelines do exist for the use of litterbags for the risk assessment of plant protection products in soils (Knacker et al., 2003; OECD, 2006), but the suggested protocol is designed specifically to test the safety of chemicals and not to consider soil organisms of different sizes. In the recent years, the growing interest for the use of the teabag method (Keuskamp et al., 2013) was also driven by the need for a standard method to measure decomposition. However, teabags do not address the standardization needs concerning soil organism's effect on decomposition as they prevent to take into account the effect of large organisms. Here, we provide some clues to develop a standard protocol for the use of multi-mesh litterbags in cropping systems, including bag design and litter characteristics (Table 5.2).

In an agricultural context, the standardized litterbag protocol should take into account the various agricultural practices and the duration of the experiments. Attention should be paid to the cropping calendar and the management practices that can influence the rate of litter decomposition (e.g. phytosanitary treatments, mineral and organic matter inputs) or affect the positioning of litterbags (e.g. tillage, seedling, harvest, mechanical weeding).

Table 5.2 Recommendations for the standardization of litterbag experiments measuring the effect of soil organisms on litter decomposition in cropping systems.

Parameter	Goal	Standardization	Recommendations
Mesh size	Select several size groups of soil organisms	Average number and size of mesh	Select 3 mesh sizes with an opening around 0.02 mm to include microorganisms only, 1 mm to additionally include mesofauna and 5 mm to include all soil organisms.
Bag size	Enclose the quantity of litter required to conduct measurements	Single size of bag	10 x 10 cm bags limit displacement or tearing during field management and provide space for the quantity of litter required to assess decomposition over the full length of the experiment. 20 x 20 cm bags can be selected under tropical climate as litter degrade faster.
Bag material	Construct solid, biologically inert and cheap mesh bags	Single material, closure techniques	Use nylon fabric and close the edges of bags by sewing with a nylon/polyester thread or by using stainless steel staples (or monel rustproof staples for long studies). Sewing allows solid closure with limited apertures on the edges of the bags and is cheap, but may be time consuming. Staples allow gaining time, but are more expensive and may create larger apertures on the edges of the bags.
Enclosed litter	Represent crop residues typically found in the cropping systems; select standard litter to compare different sites	Standard litter management, size of litter pieces, standard litter type	Homogenize litter within all bags, select a specific part of the plant (e.g. leaves, stems) or if mixed ensure the same ratio in each bag. Cut litter in homogeneous pieces (1 to 5 cm) as the role of soil fauna differ with the size of litter. Use wheat or maize as standard litter to ease comparison between studies.
Litter amount	Fill bags with the adapted quantity of litter	Minimum litter quantity	Fill bags with a litter quantity ensuring good contact between litter and soil, and adapted to the decomposition speed. It will depend on bag size, type of litter, duration of the experiment and context (e.g. 4 - 5 g litter in 10 x 10 cm bags under temperate climate).
Positioning of bags	Allow access to organisms living in the soil; prevent field management to displace or to tear bags	Standard field placement	Place bags in the row between crop plants (Broadbent and Tomlin, 1978) and bury them vertically to limit the impact of tillage and to observe soil organisms at a greater depth (Zwahlen et al., 2007; Miura et al., 2008). In no-till systems, bags can be set at the surface.
Duration of experiment	Observe mass loss patterns during litter decomposition	Minimum duration	At least 3 months. Set length and start of the experiment according to the cropping cycle to further avoid disturbances (Ouédraogo et al., 2004; Carlesso et al., 2019).
Sampling of bags	Collect enough data on litter mass loss	Minimum number of sampling dates	Number of samplings depends on required effort (e.g. distance to field, number of bags) and on cropping calendar. More than 3 dates if the aim is to assess degradation kinetics.
Sampling of soil biota	Survey soil fauna and assess its effect on decomposition	Collection of soil organisms	Count and identify soil organisms in bags whenever possible. Extract organisms from soil sampled near area of bag deposition to get an overview of the field density and diversity.
Supplementary advice	In order to track down litterbags, use stakes and centimetric precision GPS. Stakes tend to attract megafauna (e.g. wild boars) so the ideal is to place them further away of bags, on the same crop row.		

Depending on the management practices in place, litterbags may be buried or placed at the surface in order to limit physical disturbances and to model the placement of crop residues. Nonetheless, regardless of the management system in place, the positioning of litterbags in the field could significantly affect litter decomposition. Decomposition in bags could notably be influenced by the distribution and community composition of soil organisms at different depths (Beare et al., 1992; Ouédraogo et al., 2004). Placed on the surface, litterbags allow to better estimate the role of mesofauna, which is for a large part located in the first centimeters of the soil, but could lead to exclude some macrofauna organisms such as endogeic and anecic earthworms. On the other hand, buried litterbags will be better connected with soil microorganisms and less impacted by weather events (e.g. rain, UV), but they could be impacted by soil compaction.

A vast majority of the litterbag studies occurred in experimental sites, where it is easier to set an undisturbed area or to remove and replace bags at the time of main disturbances. Under real conditions, it is more difficult to request from farmers to set an undisturbed area, during tillage or mechanical weeding for instance, and to have a precise insight of the planning of their interventions ahead of time (e.g. date of harvest, tillage). Yet, the acquisition of more realistic estimates of the effect of soil biota on litter decomposition required conducting experiments under real conditions.

Finally, in order to assess more precisely the role of soil organism's in litter decomposition and to survey soil fauna in a wide range of agricultural contexts, the counting and identification of soil organisms should be achieved and reported with the decomposition results whenever possible.

5.5 Conclusion

This meta-analysis is the first, to the best of our knowledge, to deal with multi-mesh litterbag experiments in annually cropped soils and to assess the influence of different size classes of soil organisms on the litter decomposition rate. It highlights the essential role of macrofauna and mesofauna during organic matter decomposition in cropped soils, whereas these soils may be submitted to high physical and chemical disturbances. This fauna effect on decomposition may be influenced by various factors such as climate, soil, standing crop, type of enclosed litter, and in a lesser extent by agricultural systems. However, more litterbag studies would be required to conclude on the quantitative impact of these factors.

Given the variability of litterbag design and litter decomposition measurements, we pledge for a standardization of the litterbag method for the forthcoming studies, in particular in cropped soils. Here, we provide some guidelines to help to implement litterbag experiments while taking into account the specificities of cropped soils (i.e. high soil perturbations).

Future multi-mesh litterbag experiments should especially focus on alternative (e.g. organic or conservation agriculture) or transitioning systems and on different combinations of practices for which farmers and consumers are showing a growing interest. In addition, studies are also required on the effects of individual agricultural practices on soil organisms and litter decomposition, notably for crop rotation, mineral or organic inputs, tillage, shallow tillage, mechanical weeding and soil cover. This is necessary to determine their specific effect on the activity of different groups of soil organisms and the related impacts on the soil functioning. Finally, our understanding of the biological functioning of agroecosystems requires the identification of soil organisms and especially of their functional traits in relation to different agricultural practices and systems.

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———— Chapter 6 ————

Soil functions mediated by soil organisms in alternative cropping systems

Juliette Chassain¹, Sophie Joimel¹, Claire Chenu¹, Baptiste Coupery¹, Laure Vieubl  Gonod¹

Article in preparation

¹ Universit  Paris-Saclay, INRAE, AgroParisTech, UMR ECOSYS, 91120, Palaiseau, France

Abstract

The provision of ecosystem services by agricultural soils strongly relies on the relation between soil organisms and soil functions, which are in turn influenced by crop management practices and soil properties. However, the effects of cropping systems and practice intensity on organism-driven soil functions are still unclear. In this study, we investigate several soil functions in conventional, organic and conservation systems, either stable (≥ 7 years) or in transition (≤ 3 years), and discuss their relation to soil organisms and practice intensity. Soils were sampled at 0-10 and 10-20 cm depth to assess soil properties and soil organic carbon (SOC) stocks. Litter mass loss and macro- and mesofauna effects on litter decomposition were assessed using litterbags of three mesh sizes (5 mm, 1 mm, 48 μm) buried in plots for five months. Rates of C and N mineralization were measured over 120 days by conducting incubations of soil samples collected at 0-10 cm depth, and additionally at 10-20 cm depth for conservation systems. Soil aggregate stability at 0-10 cm depth was estimated by determining the mean-weight diameter (MWD) of aggregates.

SOC tended to be higher in stable conservation and organic systems than in other systems at 0-10 and 10-20 cm depth respectively. However, our results showed no significant effect of cropping systems on C mineralization and SOC stocks at 0-20 cm depth, nor on litter degradation and N mineralization. Litter decomposition was positively affected by higher tillage and lower pesticide treatment intensities in 48 μm bags, and by lower fertilization intensity in 1 mm bags. The macrofauna effect on decomposition was significantly higher in recent organic systems and was negatively impacted by pesticide treatment intensity. In addition, N mineralization (per kg of soil and per g of TN) tended to be the highest in stable conservation systems and the lowest in stable organic systems. The mineralized C after 120 days (per kg of soil) and the mineralization rate of the slow C fraction (per kg of soil and per g of SOC) were negatively affected by tillage and positively by pesticide treatment intensities. The mineralized N after 120 days (per kg of soil and per g of TN) was negatively affected by tillage intensity. Furthermore, soil aggregate stability was significantly higher in stable conservation than in conventional and organic systems due to benefits of low tillage intensity. Overall, even if few significant differences were observed, our results showed differences in soil functioning between stable alternative and conventional systems, and less differences when considering transitioning systems. Long-term studies may provide more insight on the effect of alternative systems, stable or in transition, on organism-driven functions in soil.

6.1 Introduction

Agricultural soils support crop production and provide many other ecosystem services such as protection against erosion, water quality and supply, climate regulation, and disease and pest control (Kibblewhite et al., 2008). These services partly result from the contribution of soil organisms to a wide variety of soil functions in relation to applied crop management practices and soil properties (Plaas et al., 2019). Indeed, soil organisms are essential drivers of many soil functions in agrosystems (Neher, 1999; Brussaard et al., 2007; Gaba et al., 2018; Chen et al., 2020). In particular, numerous organisms and their interactions are involved in organic matter decomposition (Chassain et al., 2021), nutrient cycling (Neher, 1999), C sequestration (Zhu et al., 2016) and soil aggregate formation (Crossley et al., 1992).

Soil fertility relies on organic matter decomposition and mineralization, which provide nutrients for plants. Crop residues or exogenous organic matter inputs are first broken down into smaller organic particles by various soil fauna organisms (Frouz et al., 2015; Chassain et al., 2021; Njoroge et al., 2022). These smaller particles can be assimilated by soil microorganisms and then mineralized (Beare et al., 1992; Autret et al., 2020). Soil fauna can also participate in the redistribution of organic matter in the soil profile through bioturbation or excretion of fecal pellets, and facilitate its accessibility to the soil microorganisms (Frouz, 2018).

Soil structural stability is of major importance for seed germination and root growing, air and water circulation, resistance to compaction, crusting, run-off and erosion, and hence for crop yield (Amézketa, 1999; Kibblewhite et al., 2008). Soil stability and resistance to erosion can be estimated by soil aggregate formation and stability (Le Bissonnais, 1996). Soil microorganisms promote the formation and the stabilization of soil aggregates (Lynch, 1981; Bossuyt et al., 2001; Lehmann and Rillig, 2015), while soil fauna complement their action by influencing soil aggregate formation (Frouz, 2018), as reported for earthworms (Six et al., 2002; Hedde et al., 2013) and Collembola (Siddiky et al., 2012; Maaß et al., 2015). Therefore, these different organisms play a major role in the stability of the soil structure at different spatial scales. Soil aggregation and soil structure are in turn demonstrated to affect food web interactions as they drive accesses and barriers between preys and predators (Six et al., 2002; Erktan et al., 2020).

The storage of soil organic carbon (SOC) is partly dependent on the decomposition and mineralization activities of soil organisms that regulate the outputs of SOC, which are controlled by the protection of SOC relying on the stability of soil aggregates.

However, agricultural soils are subjected to a large range of chemical and physical disturbances. In particular, intensive cropping systems are responsible for strong soil disturbances that may have important impacts on soil organisms and the functions they perform in soil (McLauchlan, 2006; Mazzoncini et al., 2010; Tsiafouli et al., 2015). Alternatives to

conventional systems, such as organic and conservation systems, aim to decrease the impact of crop production on soil through lower physical or chemical disturbances. Organic farming was reported to benefit the abundance of soil fauna and litter decomposition (Domínguez et al., 2014; Sereda et al., 2015) and to increase C and N mineralization rates (Gunapala and Scow, 1998) and SOC stocks (Gattinger et al., 2012) compared to conventional systems. On the other hand, conservation systems were reported to promote higher microbial biomass, bacterial and fungal abundance (Palm et al., 2014; Henneron et al., 2015) and a higher soil stability with higher aggregate stability due to no-tillage (Conti, 2015; Sekaran et al., 2021). However, recent investigations found similar litter decomposition rates in conservation and conventional systems (Cassani et al., 2021) and similar mineralization rates in organic, conservation and conventional systems (Autret et al., 2016, 2020). In addition, Govaerts et al. (2009) showed in a previous review that studies reported alternatively positive, negative or neutral effects of conservation and no-tillage agriculture on SOC stocks. Therefore, further studies are required on the simultaneous assessment of multiple soil functions to better understand the effects of alternative cropping systems on ecosystem services.

The variability of soil responses regarding cropping systems can be explained by the wide variability in the practice combinations, which are individually reported to influence soil functions. Tillage, through its action on soil physical structure, was reported to be a main factor influencing soil organisms and the delivery of soil functions. In particular, previous studies observed a decrease in litter decomposition (Reddy et al., 1994; Miura et al., 2008; Faust et al., 2019), C and N mineralization (Autret et al., 2020) and aggregate stability (Bottinelli et al., 2017) under tillage compared to no-tillage. Yet, several studies found opposite results, such as increased litter decomposition under tilled systems (Domínguez et al., 2010) and decreased C mineralization under no-tillage (Balesdent et al., 2000). In contrast, fertilization was observed to influence soil functioning, but with effects that depended on the dose, number of applications and type of fertilizer, as reported for litter decomposition (Knorr et al., 2005) and organic carbon storage (Autret et al., 2016). In addition, higher aggregate stability was observed with organic rather than with mineral inputs (Bottinelli et al., 2017). Lastly, crop protection treatments such as insecticides were reported to lower the density of decomposer organisms and the rate of litter decomposition (Pearsons and Tooker, 2021).

All of this demonstrates that cropping systems and practices have highly variable and complex effects on soil functions. However, few studies assessed soil functioning for several alternative cropping systems and practices at the same time. Knowledge is also missing on the changes occurring during the transition from conventional to alternative systems (Houben et al., 2018; Sekaran et al., 2021). Furthermore, several soil functions are rarely assessed within the

same study (Gamfeldt et al., 2008; Reiss et al., 2009), and most of the previous experiments on multiple soil functions were conducted in laboratory conditions or on experimental sites.

Here, we studied the effects of alternative cropping systems (organic and conservation agriculture, stable or in transition) compared to conventional systems, on different soil functions driven by soil organisms (litter decomposition, C and N mineralization, soil aggregate stability). This study was based on the soils of 21 plots owned by farmers, with measurements of functions performed either directly on field or on soil samples in laboratory conditions, hence accounting for the variability of agricultural practices in real conditions. We hypothesized that litter decomposition and soil structural stability would be higher under systems with lower soil physical (i.e. conservation systems) and chemical (i.e. organic systems) disturbances. In addition, we presumed that C and N mineralization may differ between conventional and organic and conservation systems due to different organic matter contents, and that this difference may depend on the stage of the system and of the soil depth.

6.2 Materials and methods

6.2.1 Study sites and agricultural practices

The soil sampling and field experiments were conducted in 21 plots owned by farmers and located in the Paris Basin, France (cf. General introduction). All plots were cropped with winter wheat in 2020-2021 and with various crops or cover crops in 2021-2022 (Table 6.1). Soils have a silty to clayed type, with a pH ranging from 5.6 to 8.1 and a bulk density varying between 1.1 and 1.5 g.cm⁻³ at 0-10 cm depth (Table 6.1).

Plots were grouped into six cropping systems namely: i) stable systems (≥ 7 years) under conventional (Conv, n = 6 plots), conservation (CA, n = 3) or organic agriculture (OA, n = 3), and ii) systems in transition (2-3 years) from conventional to organic (Conv-OA, n = 3), conventional to conservation (Conv-CA, n = 3) or conservation to organic agriculture (CA-OA, n = 3) (Table 6.1).

Information on practices applied to each plot were investigated by conducting a survey among farmers and used to compute primary indicators and composite indexes of practice intensity following Büchi et al. (2019) (cf. General introduction). Composite indexes represented tillage, pesticide treatment and fertilization intensities. The values of indicators and indexes are presented in Table 6.2.

Table 6. 1 Location, crop, cropping system and soil properties of the study plots. Clay, silt and sand contents and pH are given for samples at 0-10 cm depth. BD, SOC and TN are given at both 0-10 cm and 10-20 cm depth. Year since conversion corresponds to the number of years since conversion to the current system in 2020.

Plot	Region	Crop 2020	Crop 2021	System	Age	Clay	Silt	Sand	pH	BD ₀₋₁₀	BD ₁₀₋₂₀	SOC ₀₋₁₀	SOC ₁₀₋₂₀	TN ₀₋₁₀	TN ₁₀₋₂₀
						g.kg ⁻¹	g.kg ⁻¹	g.kg ⁻¹		g.cm ⁻³	g.cm ⁻³	g.kg ⁻¹	g.kg ⁻¹	g.kg ⁻¹	g.kg ⁻¹
A1	Eure-et-Loir	wheat	wheat	Conv-CA	3	151.3	474.3	55.7	6.8	1.3	1.4	11.3	8.9	1.09	0.92
A2	Essonne	wheat	cover	CA	17	210.3	381.3	118.7	6.3	1.3	1.5	19.4	11.1	1.79	1.14
A3	Essonne	wheat	wheat	OA	20	157.7	409.0	196.3	6.5	1.4	1.5	10.1	11.1	1.02	1.10
A4	Yvelines	wheat	bare	OA	19	426.3	236.7	93.3	7.9	1.1	1.2	19.9	19.0	2.27	2.01
A5	Eure-et-Loir	wheat	barley	Conv	20	206.0	448.0	52.0	6.8	1.3	1.5	14.2	11.0	1.34	1.12
A6	Essonne	wheat	bare	CA-OA	2	277.3	377.7	28.7	6.6	1.3	1.4	12.1	12.2	1.27	1.31
A7	Yvelines	wheat	rye	Conv-OA	2	172.3	481.3	63.0	6.6	1.2	1.3	12.9	13.5	1.15	1.24
A8	Essonne	wheat	mustard	CA	7	192.7	363.7	182.7	5.6	1.5	1.5	13.9	9.7	1.26	0.94
A9	Yvelines	wheat	rapeseed	Conv	20	208.0	402.3	187.3	6.8	1.3	1.4	10.7	10.5	1.09	1.07
A10	Eure-et-Loir	wheat	alfalfa	Conv	20	219.7	441.7	46.7	7.2	1.3	1.3	12.1	11.5	1.20	1.15
A11	Yvelines	wheat	cover	OA	20	252.7	350.0	120.3	7.3	1.2	1.2	15.6	16.7	1.58	1.64
A12	Yvelines	wheat	cover	Conv	20	221.3	352.0	164.3	7.4	1.3	1.3	13.5	13.0	1.38	1.35
A13	Yvelines	wheat	rye, lentil	CA-OA	2	248.3	411.7	75.3	6.9	1.2	1.4	19.3	15.5	1.83	1.52
A14	Yvelines	wheat	cover	Conv-CA	3	175.0	385.0	119.7	6.8	1.2	1.1	18.5	14.6	1.69	1.41
A15	Yvelines	wheat	wheat	Conv-CA	3	193.3	395.3	91.7	7.0	1.4	1.5	16.3	13.7	1.54	1.33
A16	Essonne	wheat	mustard	Conv	20	284.3	338.0	66.7	8.1	1.4	1.5	14.0	12.1	1.43	1.28
A17	Yvelines	wheat	rapeseed	CA	10	228.0	349.0	203.3	8.1	1.2	1.4	21.0	18.1	1.95	1.67
A18	Yvelines	wheat	bare	Conv-OA	3	195.7	454.7	68.3	6.8	1.2	1.4	13.8	14.3	1.22	1.22
A19	Yvelines	wheat	clover	Conv-OA	2	279.7	264.0	262.0	7.7	1.3	1.3	13.7	15.9	1.44	1.59
A20	Eure-et-Loir	wheat	cover	CA-OA	2	204.0	407.0	49.3	7.5	1.4	1.4	9.8	11.9	0.99	1.20
A21	Eure-et-Loir	wheat	barley	Conv	20	340.0	226.3	238.0	8.0	1.2	1.3	19.0	16.4	1.82	1.68

Conv: conventional agriculture, CA: conservation agriculture, OA: organic agriculture, Conv-CA: transition from conventional to conservation (≤ 3 years), Conv-OA: transition from conventional to organic (≤ 3 years), CA-OA: transition from conservation to organic (≤ 3 years), BD: bulk density, SOC: soil organic carbon, TN: total soil N content.

Table 6. 2 Primary indicators and composite indexes of practice intensity used to characterize cropping systems. Values are presented for 2020 and 2021. Composite indexes are given in bold. Primary indicators were computed for practices occurring in one year or in five years for nbCrop, soilP, nbOrg, resExp. Indexes were adapted from Büchi et al. (2019).

Name	Description	Mean value in cropping system (2020 ; 2021)					
		Conv	CA	Conv-CA	OA	Conv-OA	CA-OA
nbCrop	Crops in the rotation	3.2 ; 3.5	3.3 ; 3.7	3.7 ; 3.7	3.7 ; 3.7	3 ; 3.7	3.3 ; 3.7
nbDTill	Deep tillage interventions (> 15 cm)	0.7 ; 0.7	0 ; 0	0 ; 0	1.7 ; 1.3	1.7 ; 1.3	1 ; 0.3
nbSTill	Surface tillage interventions (< 15 cm)	5.3 ; 4	0.7 ; 0	0.3 ; 0.3	6.3 ; 7.7	3.7 ; 7	7 ; 8.3
soilP	Type of tillage (plough = 0.5, no-till = 5)	2 ; 2	4.9 ; 4.9	3.8 ; 4.1	1.3 ; 1.3	1.5 ; 1.5	2.3 ; 2.3
nbHerb	Herbicide treatment applications	5.2 ; 7.8	6.3 ; 6	5.3 ; 6.3	0 ; 0	0 ; 0	0 ; 0
nbFung	Fungicide treatment applications	0.8 ; 1.8	0.3 ; 1.7	1 ; 3.3	0 ; 0	0 ; 0	0 ; 0
nbInsect	Insecticide treatment applications	1.3 ; 0.8	0 ; 0.7	2.3 ; 2	0 ; 0	0 ; 0	0 ; 0
nbOrg	Organic matter inputs	0.8 ; 0.8	0.7 ; 0.7	1 ; 1	5 ; 5	3.3 ; 3.3	2.3 ; 3
qminN	Mineral N fertilizers inputs (kg N.ha ⁻¹)	128.9 ; 198	109.3 ; 130.4	69.1 ; 187.5	0 ; 0	0 ; 0	0 ; 0
resExp	Residues exportation	0.3 ; 0.3	0 ; 0	2.3 ; 2.3	1 ; 1	2.3 ; 2.3	0 ; 0.3
Itill	Tillage intensity	0.5 ; 0.4	0 ; 0	0.1 ; 0.1	0.7 ; 0.7	0.6 ; 0.6	0.6 ; 0.4
Itreat	Pesticide treatment intensity	0.7 ; 0.8	0.6 ; 0.6	0.8 ; 0.8	0 ; 0	0 ; 0	0 ; 0
Ifertil	Fertilization intensity	0.3 ; 0.3	0.2 ; 0.2	0.4 ; 0.5	0.4 ; 0.4	0.4 ; 0.4	0.1 ; 0.2

Conv, CA and OA: conventional, conservation and organic agriculture; Conv-CA, Conv-OA and CA-OA: initial-recent system transitions.

6.2.2 Soil sampling and physico-chemical analyses

Soils were sampled in autumn 2020, minimum one week after wheat sowing. On each plot, three replicates were defined, spaced from each other by 25 m and located 25 m apart from the field margins and outside of tractor traffic tracks. Composite soil samples were collected with an auger at 0-10 and 10-20 cm depth by mixing eight soil cores for each replicate. A fraction of sampled soils was sieved at 4 mm, air dried and analyzed for the main soil physico-chemical properties by the INRAE Laboratory of Arras. The remaining soil was stored at 4°C. Bulk density was assessed by collecting soil cores (10 cm in diameter, 10 cm high) at 0-10 and 10-20 cm depth, then dried (105°C, 48h) and weighed. We estimated the stock of soil organic carbon (SOC) in each sample, such as:

$$SOC_{stock} = SOC \times S2 \times d \times BD$$

where SOC_{stock} is the stock of soil organic carbon (t C.ha⁻¹), SOC is the SOC content in the fine soil fraction (kg.t⁻¹), S2 = 10 000 m², d is the considered depth (m) and BD is the bulk density (t.m⁻³). As we did not take into account the proportion of rock fragments, our value can be considered as an estimate of the maximum stock of organic carbon in soil (Poeplau et al., 2017).

6.2.3 Litterbag experiment

The effects of cropping systems and soil fauna on litter decomposition were studied with a litterbag experiment. Experimental design and litterbag characteristics were determined following our previous literature review on litterbag experiments in agricultural areas (Chassain et al., 2021).

Maize leaves were collected on standing plants right before the harvest of autumn 2019, dried at 40°C and stored. Litterbags (10 x 10 cm) were constructed with 5 mm, 1 mm and 48 µm nylon meshes and filled with 4 g (0.001 g precision) of 1 cm² pieces of dried (40°C, 48h) maize leaves. The bags were closed by sewing, annotated and weighed. Three litterbags of each mesh size were placed on each plot between October 19th and December 2nd 2020. The bags were buried vertically at 0-10 cm depth and spaced from approximately 10 cm within the same crop row. A total of 189 litterbags were distributed among the 21 plots.

The bags were retrieved after five months, between March 22nd to May 5th 2021, placed in individual zip plastic bags, weighed after returning to the laboratory and kept at 4°C. All bags were dried (40°C, 48h) and weighed to assess water content at retrieval. Then, the litterbags were opened and litter was thoroughly rinsed under tap water over a 50 µm sieve in order to remove soil and roots. The litter and mesh were dried (40°C, 48h) and weighed to assess the remaining mass of litter. Finally, the ash free dry weight (AFDW) of litter was assessed by burning 1 g of litter

from each bag in a muffle furnace (550°C, 4h). Litter mass loss was estimated as the difference between the initial and the remaining AFDW of litter.

The fauna effect on litter decomposition was assessed as the additional mass loss resulting from the inclusion of a group of soil fauna in bags compared to the mass loss in bags where this group was excluded, such as:

$$FE = \%ML_L - \%ML_S$$

where FE is the fauna effect (macrofauna, mesofauna or combined), $\%ML_L$ and $\%ML_S$ are respectively the percentage of mass loss in larger (5 mm or 1 mm) and smaller (1 mm or 48 μ m) mesh bags. The litter decomposition was due to the activity of microorganisms and microfauna in 48 μ m mesh bags. The mesofauna effect was the difference in mass loss with 1 mm and 48 μ m mesh, the macrofauna effect was the difference between 5 mm and 1 mm mesh, and the combined effect of macro- and mesofauna was the difference between 5 mm and 48 μ m mesh.

6.2.4 C and N mineralization in laboratory conditions

All soil samples collected at 0-10 cm depth (i.e. three replicates from each plot), and samples collected at 10-20 cm depth for CA and Conv-CA plots, were incubated at 20°C and 80% of pF 2.5 for 120 days. Prior to incubation, the fresh soil samples were sieved at 4 mm and the water content corresponding to pF 2.5 (field capacity) was determined using Richard's press. A pre-incubation of one week was conducted in order to reactivate soil microorganisms and to prevent a mineralization flush at the beginning of measurements.

For C mineralization, 50 g of soil were disposed in a 0.5 L glass jar hermetically closed, in the presence of water to prevent soil drying. Measurements of CO₂ emissions were performed at eight dates (1, 3, 7, 14, 30, 60, 90, 120 days after the start of the incubation). For each date, a sample of air was extracted from each jar and injected into a gas chromatograph MICRO-GC (Agilent 3000A, Santa Clara, CA, USA) to measure CO₂ concentration. Flushes of CO₂ with CO₂-free air in jars were performed at the beginning of the incubation (day 0) and after several measurements (days 7, 14, 30, 60 and 90). Soil moisture was monitored over time by weighing, and readjusted when necessary.

For N mineralization, 25 g of soil were disposed directly in 40 mL plastic vials. Extraction of mineral N was performed at seven dates (0, 7, 14, 30, 60, 90, 120 days after the start of the incubation). Mineral N was extracted by adding 50 mL of KCl solution (1 M) to the vials. Vials were placed under agitation for one hour, centrifuged, and filtered (1.2 μ m) in clean 40 mL vials placed at -20°C before measurement. Soil humidity was readjusted for the remaining samples at all extraction dates. Mineral N concentration was measured by continuous flow-colorimetry (Scalar Analytical, The Netherlands).

The cumulative amounts of mineralized C and N were expressed as absolute mineralization per unit of soil mass (mg C.kg soil⁻¹ or mg N.kg soil⁻¹) and as specific mineralization per unit of soil organic carbon (SOC) or soil total nitrogen (TN) (mg C.g SOC⁻¹ or mg C.g TN⁻¹).

Models were fitted on the results to assess C and N mineralization kinetics following the formula used in Autret et al. (2020). The cumulated mineralized C was fitted to a “zero-first order” model:

$$C = R \times (1 - e^{-kt}) + S \times t$$

where C is the absolute or specific quantity of mineralized C (mg C.kg soil⁻¹ or mg C.g SOC⁻¹), t is the incubation time (day), k is the mineralization rate constant (day⁻¹), R is the rapidly mineralized C pool (mg C.kg soil⁻¹ or mg C.g SOC⁻¹) and S is the mineralization rate of the slow mineralizing C pool (mg C.kg soil⁻¹.day⁻¹ or mg C.g SOC⁻¹.day⁻¹). Models were fitted for each plot, using the three replicates, and model parameters were estimated using the non-linear least square (nls) function of the *stats* package in R (R Development Core Team 2020).

The cumulated mineralized N was estimated for each plot using a linear model:

$$N = D \times t$$

where N is the absolute or specific quantity of mineralized N (mg N.kg soil⁻¹ or mg N.g TN⁻¹) and D is the absolute or specific mineralization rate (mg N.kg soil⁻¹.day⁻¹ or mg N.g TN⁻¹.day⁻¹).

6.2.5 Measurement of aggregate stability

Soils were sampled in autumn 2021 by extracting a 20 cm wide and 10 cm deep block of soil with a spade. Samples were handled with care and placed in a plastic box to avoid modifying soil physical properties. The soil was air-dried for one week and larger aggregates were regularly broken by hand. Soil aggregates with a 3.15-5 mm diameter were selected by sieving and oven dried (40°C, 24h) to remove remaining water.

Measurements of soil aggregate stability were performed following the methodology of Le Bissonnais (1996) and the French norm (Norme NF X31-515, 2005). We selected the slow wetting treatment that simulates gentle rain in field conditions and better discriminates unstable soils (Le Bissonnais, 1996). For each sample, 5 to 5.2 g of aggregates were weighed (0.001 g precision) and placed for 30 min on a filter paper on the top of Bultex foam soaked with water. Saturated aggregates were transferred on a 50 µm sieve immersed in 90% ethanol. Helicoidal movement was applied five times to the sieve with a Hénin apparatus to collect the soil fraction larger than 50 µm. The collected fraction was dried (105°C, 48h) and sieved on a column of six sieves (2000, 1000, 500, 200, 100 and 50 µm). Each collected fraction was weighed to estimate its mass percentage. The weight of each fraction was corrected by removing the mass of small stones and plant debris sorted by aggregates dissolution in water and sieving. Aggregate stability was

assessed by calculating the mean weight-diameter (MWD) (Kemper and Rosenau, 1986) and accounting for the weight correction:

$$MWD = X_1 \frac{W_1 - W_{corr}}{W_{tot} - W_{corr}} + \sum_{i=2}^n X_i \frac{W_i}{W_{tot} - W_{corr}}$$

where X_1 and W_1 are respectively the average diameter and the weight of the largest size fraction, X_i and W_i are the average diameter and weight of the size fraction i , W_{tot} is the total weight of the sample and W_{corr} is the weight of stones and plant debris.

6.2.6 Statistical analyses

Statistical tests were conducted to assess the effect of cropping systems and practice intensity on measured soil functions. Prior to the analyses, sub-samples were pooled by plots by averaging the values of the three replicates. The normality and homogeneity of variances were tested with a Shapiro-Wilk test ($\alpha > 0.05$) and a Bartlett test ($\alpha > 0.05$).

Differences in litter mass loss, fauna effect, mineralized C and N on day 120, parameters of C and N mineralization rates, aggregate stability, soil properties (SOC, TN) and SOC stocks between cropping systems were assessed with an analysis of variance (ANOVA) followed by a Tukey HSD post-hoc test. The relation between the previously mentioned parameters and the value of the tillage, treatment and fertilization intensity indexes were tested using a linear model. In addition, differences in the parameters of C and N mineralization were compared between 0-10 and 10-20 cm depth for CA and Conv-CA systems and between CA and Conv-CA systems at 10-20 cm depth using Student's t-test.

All the analyses were performed using R software version 3.6.3 (R Development Core Team 2020) and the *stats* (R Development Core Team 2020) and *ade4* (Dray and Dufour, 2007) packages.

6.3 Results

6.3.1 Soil properties

The soils of CA plots were significantly different from the soils of the other systems at 0-10 cm depth (cf. General introduction, Figure I.12). In addition, CA and Conv-CA plots differed from Conv plots at both 0-10 and 10-20 cm depth.

We found no significant effect of cropping systems on SOC or TN at 0-10 or 10-20 cm depth (ANOVA, $P > 0.05$). However, trends showed that the SOC content was higher in CA (18.1 ± 3.8 g.kg⁻¹) than in OA (15.2 ± 4.9) and Conv (13.9 ± 2.8) at 0-10 cm depth, and in OA (15.6 ± 4.1) than in CA (13.0 ± 4.5) and Conv (12.4 ± 2.1) at 10-20 cm depth. In addition, SOC tended to be higher at 0-10 than at 10-20 cm depth in CA but not in Conv-CA. SOC and TN were not influenced by tillage or pesticide treatment intensities, but increased at 10-20 cm depth with an increase in the number of organic matter inputs over 5 years (cf. General introduction, Table I.7).

Table 6. 3 Effect of practice intensity on (a) litter decomposition, (b) C and N mineralization at 0-10 cm depth, (c) soil aggregate stability and (d) SOC stocks. *t*-value and *P*-value were obtained using linear model. Bold values indicate significant effects ($P < 0.05$ *, < 0.01 **, < 0.001 ***). Primary indicators with a significant ($P < 0.05$) positive (+) or negative (-) effect are reported.

	Itill		Itreat		Ifertil		Indicators
	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	
(a) Litter decomposition							
Mass loss 5 mm	1.28	0.221	-2.12	0.051	-1.71	0.108	(-) nbHerb
Mass loss 1 mm	0.91	0.376	-0.93	0.364	-2.94	0.009**	-
Mass loss 48 µm	2.31	0.032*	-2.39	0.027*	-1.98	0.062	(+) nbSTill ; (-) nbHerb
Macrofauna effect	1.20	0.248	-2.44	0.028*	0.57	0.576	-
Mesofauna effect	-0.51	0.613	0.51	0.617	-1.64	0.119	-
Combined effect	0.39	0.699	-1.11	0.284	-0.93	0.367	-
(b) C & N mineralization							
absolute Cmin120	-3.42	0.003**	2.31	0.032*	-0.04	0.969	(+) soilP, nbHerb, qminN ; (-) nbDTill, nbOrg
specific Cmin120	-1.27	0.217	1.38	0.185	-0.96	0.349	(+) qminN ; (-) nbOrg
absolute k	-0.92	0.369	0.32	0.755	0.73	0.477	-
absolute R	0.34	0.741	-0.13	0.899	-0.98	0.341	-
absolute S	-3.39	0.003**	3.06	0.007**	0.57	0.575	(+) soilP, nbHerb, qminN ; (-) nbDTill
specific R	0.60	0.555	-0.44	0.665	-1.84	0.083	-
specific S	-2.41	0.028*	2.47	0.025*	-0.12	0.903	(+) nbHerb, qminN ; (-) nbDTill, nbOrg
absolute Nmin120 & D	-3.87	0.001**	1.91	0.072	0.48	0.634	(+) soilP, nbHerb ; (-) nbDTill, nbSTill
specific Nmin120 & D	-3.01	0.007**	1.53	0.142	0.48	0.641	(+) soilP, qminN ; (-) nbDTill, nbSTill, nbOrg
(c) Aggregate stability							
MWC	-3.48	0.002**	0.94	0.358	-0.55	0.586	(+) soilP ; (-) nbDTill, nbSTill
Aggregates > 2mm	-3.48	0.002**	0.95	0.357	-0.62	0.543	(+) soilP ; (-) nbDTill, nbSTill
(d) SOC stocks							
SOCstock ₀₋₁₀	-1.70	0.105	0.66	0.516	0.64	0.528	-
SOCstock ₁₀₋₂₀	0.40	0.697	-0.59	0.560	0.82	0.421	(+) nbOrg

Itill: tillage intensity index, Itreat: pesticide treatment intensity index, Ifertil: fertilization intensity index, SOC: soil organic carbon, Cmin120: mineralized C after 120 days, Nmin120: mineralized N after 120 days, k: C mineralization rate constant, R: rapidly mineralizing C pool, S: mineralization rate of the slow mineralizing C pool, D: N mineralization rate, MWC: mean-weight diameter.

6.3.2 Litter decomposition

In total, 17 coarse (5 mm), 8 medium (1 mm) and 6 fine (48 μ m) mesh litterbags of 189 (i.e. 16%) were lost or torn down during the five months of the field experiment, mostly because of damage caused by wild boars or surface tillage. All bags except one fine mesh bag were lost in one of the OA plot (A4).

Litter mass loss in litterbags was similar in all cropping systems for coarse, medium and fine mesh bags after five months (Figure 6.1a). The highest mass loss was observed for coarse mesh, followed by medium and fine mesh. Mass loss was significantly higher for coarse than for fine mesh in all cropping systems except for OA due to higher variability, for medium than for fine mesh in Conv, Conv-CA, Conv-OA and CA-OA systems, and for coarse than for medium mesh in Conv-OA and CA-OA (Figure 6.1a). A decrease in pesticide treatment intensity and an increase in tillage intensity increased mass loss in fine mesh bags, and a decrease in fertilization intensity benefited mass loss in medium mesh bags with a similar trend for fine mesh bags (Table 6.3a). In addition, the water content in litterbags at their retrieval was higher in fine mesh bags than in other bags, but not significantly (Figure 6.1b).

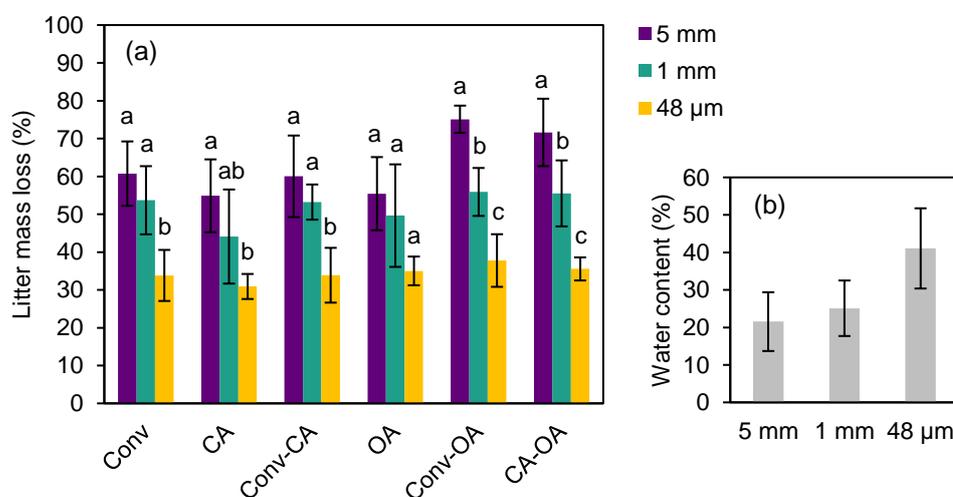


Figure 6.1 Effect of cropping systems on litter decomposition after 5 months with (a) litter mass loss in coarse (5 mm), medium (1 mm) and fine mesh (48 μ m) litterbags and (b) water content in litterbags at retrieval. Error bars are standard deviations. Different lower-case letters indicate differences in litter mass loss between bags of different mesh size within a cropping system ($P < 0.05$). Conv, CA and OA: conventional, conservation and organic agriculture; Conv-CA, Conv-OA and CA-OA: initial-recent system transitions.

Macrofauna, mesofauna and the combination of both groups foster litter decomposition in all cropping systems (Figure 6.2). The mesofauna effect was higher than the macrofauna effect in Conv and Conv-CA, while their effects were more balanced in CA and in organic systems (Figure 6.2). The combined effect of macro- and mesofauna was higher than the macrofauna effect in all systems, except for OA, and higher than the mesofauna effect in Conv-OA and CA-OA. In addition,

macro- and mesofauna effects were additive in Conv-OA and CA-OA as their combined effect was approximately the sum of their individual effect, which was not observed in the other systems (Figure 6.2). The macrofauna effect on decomposition was significantly higher in Conv-OA than in Conv, CA and Conv-CA and also than in OA and CA-OA but not significantly (Figure 6.2) and benefited from a decrease in pesticide treatment intensity (Table 6.3a). The mesofauna and combined effects were similar in all systems and did not differ according to practice intensity.

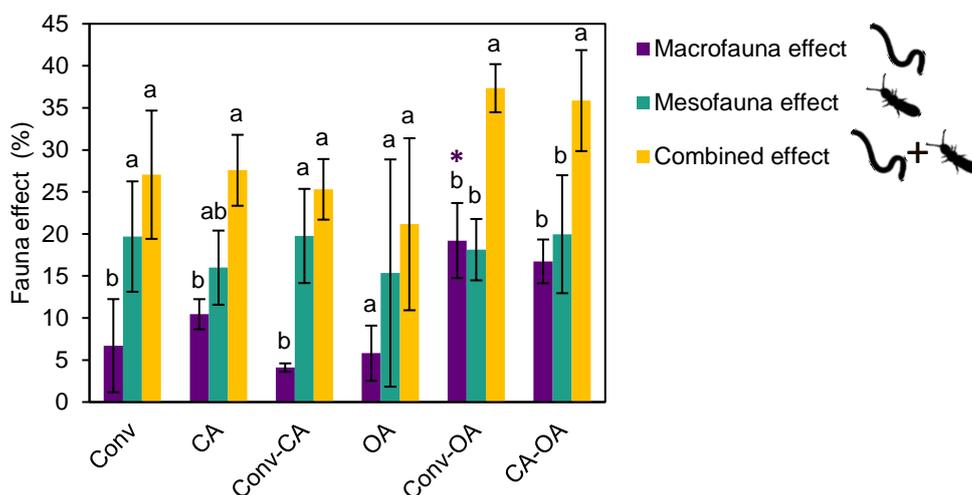


Figure 6.2 Fauna effect on litter decomposition. Macrofauna effect is the difference in litter mass loss between 5 mm and 1 mm, mesofauna effect between 1 mm and 48 μ m and combined effect between 5 mm and 48 μ m mesh bags. Error bars are standard deviations. Different lower-case letters indicate differences between fauna effects within a cropping system ($P < 0.05$). Macrofauna effect is significantly higher in Conv-OA than in Conv, CA and Conv-CA ($P < 0.05$ *) with similar trend regarding OA and Conv-OA ($P < 0.1$). Conv, CA and OA: conventional, conservation and organic agriculture; Conv-CA, Conv-OA and CA-OA: initial-recent system transitions.

6.3.3 C and N mineralization

After 120 days of incubation, the cumulative amounts of mineralized C and N did not reach a plateau and were similar in all cropping systems for both absolute (per kg of soil) and specific (per g of SOC or TN) values (Figure 6.3). However, at 0-10 cm depth, CA tended to have high absolute and specific amounts of mineralized N compared to the other systems, and Conv tended to have higher values than organic systems. CA and Conv had similar absolute amounts of mineralized C after 120 days, which tended to be higher than in OA, whereas the specific values were similar in CA and OA and lower than in Conv. Systems in transition differed from initial and corresponding stable systems, except for Conv-CA that had very similar absolute and specific amounts of mineralized C and N than Conv. In addition, the absolute mineralized C at 10-20 cm depth was significantly lower in CA than in Conv-CA (t-test, $P < 0.05$) (Figure 6.3). The absolute mineralized C and N were higher at 0-10 than at 10-20 cm depth in CA and Conv-CA, and the specific mineralized N was higher at 0-10 than at 10-20 cm depth in CA (t-test, $P < 0.05$).

A lower amount of absolute mineralized C and of both absolute and specific mineralized N were observed as tillage intensity increased (Table 6.3b). An increase in the pesticide treatment intensity had a positive effect on the absolute mineralized C (Table 6.3b). The fertilization intensity index showed no effect, but an increase in organic matter inputs had a negative effect on the specific mineralized C and N (Table 6.3b).

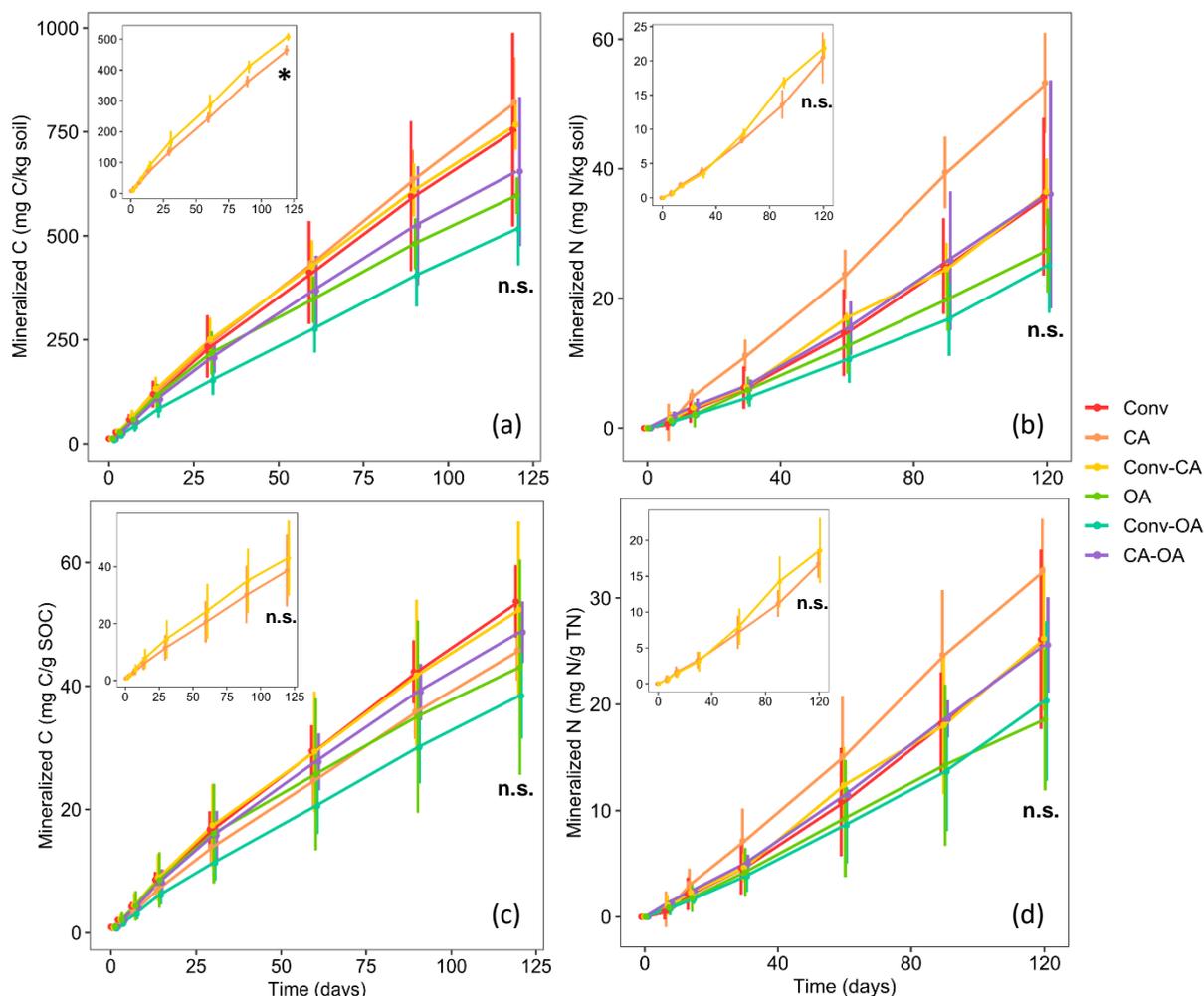


Figure 6. 3 Kinetics of mineralized C and N in cropping systems expressed as (a), (b) mineralized C or N per kg of soil and (c), (d) mineralized C or N per g of initial SOC or TN at 0-10 cm depth. Smaller graphics represent the results obtained at 10-20 cm depth for conservations systems. Points represent mean values per cropping system. Error bars are standard deviations. Significant differences at 120 days are indicated ($P < 0.05$ *; n.s.: non-significant). Conv, CA and OA: conventional, conservation and organic agriculture; Conv-CA, Conv-OA and CA-OA: initial-recent system transitions.

The models of C and N mineralization kinetics fitted well to our data. Results obtained for the absolute mineralized N at 120 days and for the N mineralization rate were the same (i.e. linear model). We observed no significant difference between cropping systems regarding the fitted parameters of the C kinetics (Table 6.4). However, the absolute mineralization rate of the slow mineralizing C pool (S) was higher at 0-10 than at 10-20 cm depth in CA and Conv-CA (t-test, $P <$

0.05). In addition, S was lower as tillage intensity and organic matter inputs increased and as pesticide treatment intensity decreased for absolute and specific C mineralization (Table 6.3b).

Table 6. 4 Absolute and specific C and N mineralization rates in soil for different cropping systems. Samples were collected at 0-10 cm depth in all plots and at 10-20 cm depth in conservation systems only. Parameters of C mineralization (k, R, S) were obtained with curve fitting. k is the mineralization rate, R is the rapid mineralizing C pool and S is the mineralization rate of the slow mineralizing C pool. D is the N mineralization rate.

Cropping system	Depth	Absolute Cmin			Specific Cmin		Absolute Nmin	Specific Nmin
		k (d ⁻¹)	R (mg C.kg soil ⁻¹)	S (mg C.kg soil ⁻¹ .d ⁻¹)	R (mg C.g SOC ⁻¹)	S (mg C.g SOC ⁻¹ .d ⁻¹)	D (mg N.kg soil ⁻¹ .d ⁻¹)	D (mg N.g TN ⁻¹ .d ⁻¹)
Conv	0-10	0.037 ± 0.010	131 ± 47	5.4 ± 2.1	9.9 ± 4.6	0.38 ± 0.07	0.30 ± 0.10	0.22 ± 0.07
CA	0-10	0.082 ± 0.051	67 ± 60	6.3 ± 1.4	5.1 ± 4.7	0.34 ± 0.00	0.44 ± 0.06	0.27 ± 0.04
	10-20	0.109 ± 0.097	37 ± 28	3.6 ± 0.3	3.4 ± 2.8	0.30 ± 0.09	0.17 ± 0.03	0.14 ± 0.02
Conv-CA	0-10	0.067 ± 0.043	117 ± 85	5.5 ± 0.4	8.5 ± 7.3	0.37 ± 0.07	0.30 ± 0.04	0.22 ± 0.06
	10-20	0.112 ± 0.128	86 ± 75	3.5 ± 0.5	7.9 ± 8.6	0.30 ± 0.05	0.18 ± 0.01	0.15 ± 0.04
OA	0-10	0.059 ± 0.032	135 ± 94	3.9 ± 0.4	11.3 ± 9.7	0.27 ± 0.07	0.23 ± 0.05	0.15 ± 0.06
Conv-OA	0-10	0.089 ± 0.057	41 ± 29	4.0 ± 0.6	3.1 ± 2.2	0.30 ± 0.04	0.21 ± 0.06	0.17 ± 0.06
CA-OA	0-10	0.031 ± 0.010	124 ± 41	3.6 ± 0.6	11.8 ± 5.4	0.33 ± 0.01	0.30 ± 0.15	0.21 ± 0.04

Conv, CA and OA: conventional, conservation and organic agriculture, Conv-CA, Conv-OA, CA-OA: initial-recent system transitions, Cmin: C mineralization, Nmin: N mineralization, SOC: soil organic C, TN: total soil N content.

6.3.4 Structural stability

The soil aggregate stability, as represented by the MWD, increased significantly with soil organic matter (ANOVA, $P < 0.05$). In addition, the MWD was significantly higher in CA than in Conv and OA (Figure 6.4a). This was mostly due to a higher proportion of aggregates with a size greater than 2 mm (after slow wetting treatment) in CA systems (Figure 6.4b). Both MWD and the proportion of aggregates of more than 2 mm decreased as tillage intensity increased (Table 6.3c).

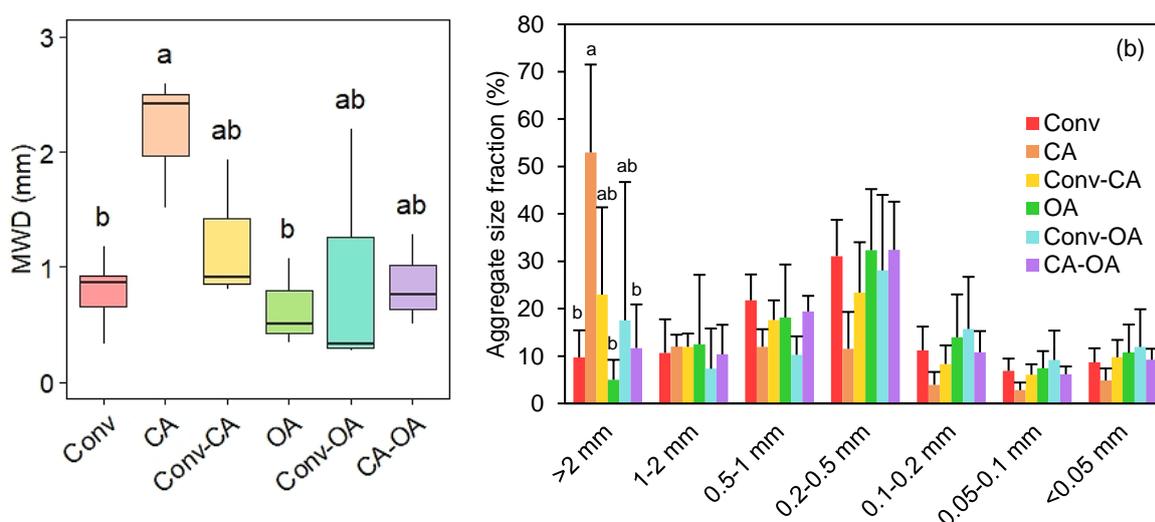


Figure 6. 4 Aggregate structural stability represented by (a) the mean weight-diameter (MWD) and (b) the percentage of the aggregate size fractions. Different lower-case letters indicate significant differences between cropping systems according to ANOVA ($P < 0.05$). Conv, CA and OA: conventional, conservation and organic agriculture; Conv-CA, Conv-OA and CA-OA: initial-recent system transitions.

6.3.5 SOC stocks

The SOC stock was similar in all cropping systems at 0-10 and 10-20 cm depth (Figure 6.5). In addition, it was not significantly influenced by practice intensity (Table 6.3d). However, we observed a positive relation between SOC stock at 10-20 cm and the number of organic matter inputs over five years (Table 6.3d).

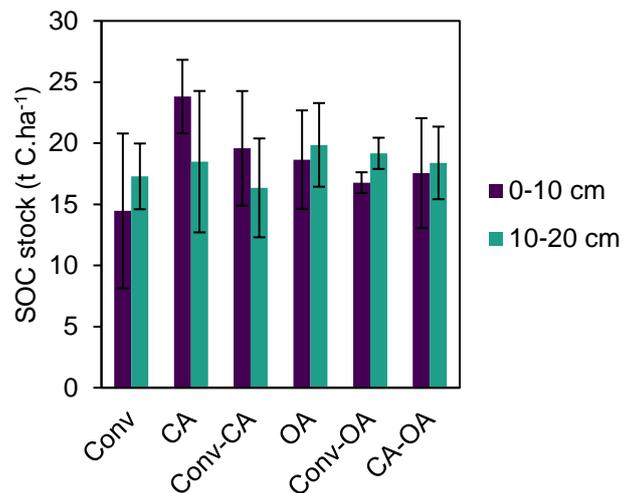


Figure 6. 5 Stocks of soil organic carbon (SOC stock) at 0-10 and 10-20 cm depth. Conv, CA and OA: conventional, conservation and organic agriculture; Conv-CA, Conv-OA and CA-OA: initial-recent system transitions.

6.4 Discussion

6.4.1 Effects of cropping systems and soil organisms on litter decomposition

Litter decomposition expressed with litter mass loss was similar in all studied cropping systems, in line with previous studies (Cassani et al., 2021). Litter can be degraded by different soil organisms that are more or less abundant and diverse (Chapter 1 to 4) and more or less active in the studied systems. Considering practices, we observed that high tillage and low pesticide treatment intensities may increase litter decomposition by microorganisms (i.e. higher mass loss in fine mesh bags). It could be a consequence of the effect of pesticides (Beare et al., 1993; Bünemann et al., 2006) and tillage (Anderson et al., 2017; Le Guillou et al., 2019; Wang et al., 2021) on the soil microbial community and microbial diversity (see Chapter 3), which may in turn affect microbial activity of litter degradation. Tillage mixes organic matter with soil, thus increasing its accessibility and degradation by microorganisms. Inversely, no-tillage is reported to increase soil compaction, which could limit the effect of soil organisms on litter decomposition. Compaction has limited direct effects on microorganisms owing to its small effect on

microporosity (Mamy et al., 2020). However, it decreases litter decomposition through negative effects on soil macrofauna, in particular on earthworms (Domínguez et al., 2010), which have cascading effects on the dissemination and regulation of soil microbial communities. In addition, we showed that fertilization intensity also affected the decomposition in medium mesh bags including both microorganisms, micro- and mesofauna, which contrast with studies reporting limited effect of fertilization type on decomposition and microbial communities (Knorr et al., 2005; Ge et al., 2021).

The estimation of the fauna effects (i.e. difference in litter mass loss between bags) helped to better understand the role of soil meso- and macrofauna in decomposition under different cropping systems. Our results regarding fauna effects on litter decomposition are in agreement with the conclusions of our previous meta-analysis (Chassain et al., 2021). Indeed, in this latter study, we reported similar and non-additive effects of meso- and macrofauna on litter decomposition and suggested that these effects might vary depending on cropping systems. Here, litter decomposition was significantly increased in the presence of meso- and macrofauna but the proportion of their effects on litter decomposition varied according to cropping systems. In conventional and conservation systems, mesofauna played a major role in litter decomposition, whereas both meso- and macrofauna seemed to participate equally to decomposition in recent organic systems. However, this trend was only observed for recent organic systems and is difficult to confirm for the stable organic systems because of a lack of data resulting from the important loss of litterbags in one organic plot and of the high variability of results. In addition, we observed an additive effect of meso- and macrofauna on litter decomposition in recent organic systems, contrasting with the non-additive effect reported overall for agricultural soils (Chassain et al., 2021). Moreover, the macrofauna effect on litter decomposition was higher in recent organic systems than in all other systems. This is in agreement with reported benefits of organic systems on faunal decomposers (Birkhofer et al., 2008b), in particular on Diplopoda density (see Chapter 4), which we however observed to increase in stable but not in recent organic systems. The benefits of organic systems are primarily explained by the absence of pesticides. Indeed, we observed macrofauna effect to decrease when pesticide treatment intensity increased, which conforms to previously reported negative effects of pesticides on macrofauna decomposers (Wardle et al., 1999; Pearsons and Tooker, 2021).

6.4.2 Effects of cropping systems and soil organisms on C and N mineralization

As for litter decomposition observed *in situ*, the soil C and N mineralization kinetics investigated under laboratory conditions were not significantly different between cropping systems. However, we observed trends suggesting differences between alternatives systems.

In stable systems, the absolute C mineralization tended to be higher in conventional and conservation than in organic systems. However, when expressed by g of SOC, conservation and organic systems had similar but lower C mineralization than conventional systems. This suggests that various factors are controlling the C mineralization, such as the type of available organic matter, soil properties that control microbial activity (e.g. pH, clay content) or the presence of different organisms (Autret et al., 2020). In our study, different types of organic matter dominate conservation (i.e. crop residues) and organic systems (i.e. exogenous organic matter inputs). In addition, soil properties were observed to drive microbial community composition (see Chapter 3), which may in turn influence their activity in decomposition and mineralization. Besides, we observed that conservation and organic systems benefited different groups of soil fauna (Chapter 4), which may indirectly affect mineralization. The differences in available organic matter and communities of soil organisms may equilibrate and conduct to similar C mineralization in alternative systems. Furthermore, the absolute and specific N mineralization tended to be superior in conservation than in conventional systems, which was in turn superior to organic systems. Therefore, conservation and organic systems respectively tended to accelerate or slow N mineralization compared to conventional systems.

In transitioning systems, absolute and specific C and N mineralization tended to vary from initial and stable systems, except for recent conservation systems, which had almost exactly the same mineralization curves than conventional systems. In addition, recent organic systems (ConvoA) tended to have similar N mineralization curves than stable organic systems. This suggests that changes in the mineralization process are occurring faster during the conversion to organic than to conservation systems, probably due to a more active management of organic matter in organic systems. In addition, stable and recent conservation systems had an overall higher C and N mineralization at 0-10 than at 10-20 cm depth, even when considered per g of SOC or TN. At 10-20 cm depth, absolute C and N mineralization was higher in recent than in stable conservation systems, but not when expressed per g of SOC. These results are related to the higher amount of organic matter at the surface in conservation systems compared to other systems, which conducts to an increase in SOC at the surface and a decrease at 10-20 cm depth. The stratification of SOC in no-tillage systems, in particular its accumulation in the first centimeters of soil, is reported to occur soon after conversion from conventional tillage (Kay and VandenBygaart, 2002), but the loss of SOC at lower depth could take a few more years, in particular regarding recent systems (2-3 years old) in our study.

Whereas tillage benefited litter decomposition, it had an overall negative effect on the absolute C mineralization and primarily affected the absolute and specific slowly degradable C fraction. Therefore, tillage may affect long-term soil fertility, which depends on the stable C fraction (i.e. slowly mineralized) rather than short-term fertility, which depend on the labile C

fraction (i.e. rapidly mineralized). In addition, low tillage intensity positively affected absolute and specific N mineralization at 0-10 cm depth. Similarly, previous studies observed that no-tillage increases both C and N mineralization rates in the surface layer of soil, but tends to have no effect on mineralization deeper in soil compared to conventionally tilled systems (Beare et al., 1994; Jacobs et al., 2010). This was explained by an increase in organic matter and particulate organic matter in the surface layer in the absence of tillage and by a less favorable soil structure in tilled soils (Autret et al., 2020). In addition, no-tillage has reportedly the potential to increase the physical protection of organic matter in soil aggregates preventing access to organic matter by microorganisms (Balesdent et al., 2000; Six et al., 2002).

Furthermore, we observed a positive effect of the intensity of pesticide treatments on the slowly degradable C fraction. Negative effects of pesticides on soil organisms could result in the release of easily degradable and mineralized C. However, this effect may also reflect the negative effect of tillage as more pesticides are used for weed management when tillage is reduced.

Conversely, organic matter inputs had a negative effect on C and N mineralization, which contrasts with previous studies reporting a higher mineralization with an increase in organic matter inputs (Carpenter-Boggs et al., 2000; Guo et al., 2019; Wang et al., 2021). It can be explained by the variable quality of applied organic matter. In our study, an important part of the inputs in the organic plots was either poultry manure or composted organic matter (i.e. green wastes, manure). Composting provides stabilized organic matter, and compost addition to soil was demonstrated to slow C mineralization (Molina-Herrera and Romanyà, 2015). Conversely, the input of manure (i.e. unstabilized organic matter) in soil reportedly increases C mineralization (Molina-Herrera and Romanyà, 2015; Guo et al., 2019). However, this could again be related to an increase in tillage intensity, as organic matter inputs are buried using deep tillage.

It should be mentioned that our incubations were performed on sieved soil samples, and that sieving was suggested to artificially increase mineralization rates compared to undisturbed soils (Balesdent et al., 2000; Kpemoua et al., 2022). In addition, incubations on a longer term could provide more insight on the effect of cropping systems on mineralization.

6.4.3 Effects of cropping systems and soil organisms on soil aggregate stability

The soil aggregate stability was observed to be higher in stable conservation systems than in stable conventional and organic systems, in line with previous studies (Chabert and Sarthou, 2020). The higher stability under conservation systems was mainly explained by the benefits of no-tillage on aggregate formation and stabilization (Sekaran et al., 2021), on the increase in soil organic matter and on the increase in microbial biomass with changes in the microbial population in the first centimeters of soil (Palm et al., 2014; Romero-Salas et al., 2021). No-tillage could

promote fungi, especially arbuscular mycorrhizal fungi that produce glomalin (Rillig, 2004). Glomalin is a major promoter of the formation of stable soil aggregate through its action as a binding agent (Liu et al., 2020). Microbial activity might be complemented by the action of soil fauna. In particular, earthworms were reported to influence the formation of large aggregates through casting and dispersal of microorganisms (Hedde et al., 2013). In accordance with this, both earthworms and aggregate stability reportedly increase under conservation compared to conventional systems (Castellanos-Navarrete et al., 2012). Collembola may also increase soil aggregation in close connection with fungi (Siddiky et al., 2012) and mostly through indirect contributions such as grazing on microorganisms and production of organic materials (i.e. pellets, necromass) acting as a nutrient source (Maaß et al., 2015). Consistently, in our study, Collembola were observed to have the highest density in stable conservation systems (Chapter 1). Lastly, the lack of difference that we observed between stable and transitioning systems may show that aggregate formation and stabilization is rather a long-term than a short-term process. However, differences could be observed as early as 6 to 8 years after conversion to no-tillage (Bottinelli et al., 2017; Sekaran et al., 2021). In our study, recent conservation systems tended to show an increase in aggregate stability compared to conventional systems, suggesting that aggregate stability may reach a similar differentiation than stable systems in a few years.

6.4.4 Influence of cropping systems on soil organic carbon stocks and ecosystems services

Soil organic carbon stocks, estimated up to 20 cm depth, were not influenced by cropping systems nor by tillage or pesticide treatment intensities. This contrasts with previous studies reporting an increase in SOC stocks in organic (Gattinger et al., 2012) or in conservation systems (Govaerts et al., 2009) compared to conventional systems. However, SOC stock at 10-20 cm depth was related to the number of organic matter inputs over the last five years, which confirms that organic matter inputs are the most efficient way to increase SOC stocks (Autret et al., 2016). However, SOC stocks rely on complex processes, as they are the results of the balance between soil C inputs and outputs (Figure 6.6). An increase in organic matter inputs, a decrease in C mineralization and an increase in soil aggregate stability, providing a better physical protection of organic matter, have the potential to increase SOC stocks.

However, under real conditions, we observed similar SOC stocks in different cropping systems although mineralization and soil aggregate stability tended to differ (Figure 6.6). Thus, we suggest that the observed differences in soil functions between cropping systems, even if not significant, may compensate one another or rely on relations that are more complex. Indeed, in conservation systems, the higher soil aggregate stability, related to a higher SOC content observed at 0-10 cm depth, may compensate for the higher mineralization rate. Conversely, these

mechanisms seem more complex in organic systems. Organic systems rely on more organic matter inputs and may promote litter decomposition by soil macrofauna and microorganisms thanks to the absence of pesticides. However, they also showed a lower soil aggregate stability and tended to have a lower mineralization rate. Organic systems combined with reduced tillage were reported to present a high potential for SOC stocks increase (Krauss et al., 2022), building upon the benefits of both alternative systems. Furthermore, alternative systems may present differences in SOC storage compared to conventional systems but exclusively on the long-term (Autret et al., 2016).

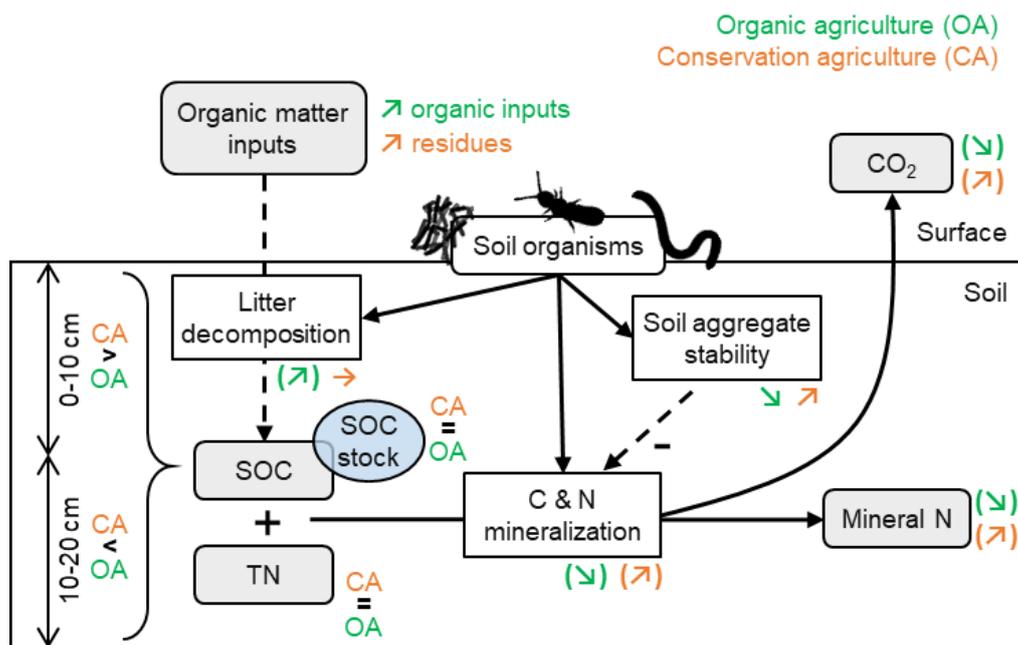


Figure 6. 6 Effect of organic and conservation systems on soil functions mediated by soil organisms. Color arrows represent the increase (↗) or decrease (↘) in functions or variables in relation to organic (green) and conservation (orange) systems. Arrows are in parenthesis when trends were observed. SOC: soil organic C, TN: total soil N content.

Overall, even if few significant differences were observed, alternative cropping systems tended to influence different soil functions in relation to their effect on soil organisms. Therefore, they have the potential to benefit different ecosystems services. Furthermore, we demonstrated that changes observed in soil functions in cropping systems are better explained by the intensity of practices and are highly dependent on their effects on soil organisms. Conservation systems promote soil aggregate stability and hence a better soil structure with benefits for crop growth, resistance to erosion and C storage through the physical protection of SOM in aggregates. They also tend to foster N mineralization, which provides more nutrients for plant growth and increases soil fertility, but may increase nitrate leaching and NH₃ emissions. These benefits are mostly related to no-tillage. Inversely, organic systems demonstrated no significant benefits on soil functions and it is difficult to assess the services they influence. The trends for higher decomposition and lower mineralization in organic systems could promote C storage, but a lower

availability of nutrients for crops with a potential decrease in yield. However, previous studies reported that the main ecosystem service promoted by organic farming is disease and pest regulation (Birkhofer et al., 2008a; Chabert and Sarthou, 2020).

Therefore, we showed stronger evidence for an increase in ecosystems services related to soil in conservation systems compared to conventional and organic systems. This is in agreement with a recent study comparing the effect of these systems on ecosystems services and reporting a higher potential of conservation systems for the provision of various ecosystems services (Chabert and Sarthou, 2020). In our study, we did not assess the effect of alternative and transitional systems on crop yield, which is major service expected from agroecosystems. Indeed, the development of alternative systems cannot only rely on environmental benefits and should take into account the potential changes in crop yield and consequences at a larger scale. However, despite being investigated in numerous studies, the effect of alternative systems on crop yield is still controversial (Seufert et al., 2012; Palm et al., 2014; Chabert and Sarthou, 2020). Crop yield can decrease to some extent in alternative systems to promote other services, but this loss should be compensated by cost savings (i.e. fuel, fertilizers) for farmers and should not put at risk food security.

6.5 Conclusion

This study investigated different organism-driven soil functions (decomposition of organic matter *in situ*, C and N mineralization in laboratory conditions, aggregates stability and effects on C storage) under conventional, stable and transitional alternative cropping systems. Soil functions are influenced by cropping systems, even if few significant effects were observed, and by the intensity of applied practices in relation to their impact on soil organisms. Therefore, our results emphasize that the effects of cropping systems on soil organisms should be considered carefully in order to develop systems that rely on strong soil biodiversity and soil functioning. Long-term investigation may provide more insight on the effect of alternative systems on soil functions and more generally on ecosystem services. However, on the short-term, the development of systems combining low chemical disturbances and reduced tillage may provide a good option to promote soil ecosystem functioning.

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———— General Discussion and Conclusion ————

The development of sustainable agroecosystems requires the promotion of cropping systems relying strongly on biodiversity and ecosystem functioning. Organic and conservation agriculture currently represent the main alternatives to intensive agriculture and more and more farmers may opt for these systems in upcoming years. However, the effects of these alternative systems on soil biodiversity and soil functioning at the short and long-term remain poorly understood. In this context, this thesis aims to provide evidence on the effects of stable and transitional alternative cropping systems on soil biodiversity and associated ecosystem services.

This work contributes to filling the previously underlined research gaps by comparing different alternative cropping systems to conventional ones as well as transitional and stable alternative systems. In addition, we considered the density and diversity of multiple groups of soil organisms, which are rarely all studied together, and their interactions within the soil food web. Furthermore, we took into account multiple soil functions and activities performed by soil organisms, informing on the provision of soil-based ecosystem services in different cropping systems. Lastly, this work further relies on practice intensity indexes, generally used by agronomists, to better understand disturbances caused by cropping systems, and on the functional traits of several groups of soil organisms (Collembola, earthworms and bacteria) to get a larger picture of soil biodiversity.

Overall, we demonstrated that alternative systems relying on low physical and chemical disturbances have the potential to influence the density and diversity of soil mesofauna, macrofauna and microorganisms (Chapters 1 to 3), the interactions of these organisms within the soil food web (Chapter 4) and the functions they drive in agricultural soils (Chapters 5 and 6).

The following section synthesizes the main results of this study and confronts our initial hypotheses, while discussing the contribution of this thesis to soil ecology in an agricultural context. It also outlines some perspectives that would be interesting to pursue this work and ends with a short opening about science outreach on soil biodiversity.

D.1 Synthesis of the main results and contribution of the PhD thesis to soil ecology

What are the effects of alternative cropping systems on soil biodiversity and soil functions?

Alternative systems are often studied independently, in comparison to conventional systems. This work compares the two main alternatives currently developed and assesses their overall benefits on the density and diversity of soil organisms and on associated soil functions compared to conventional systems. Our results show that alternative systems promote soil biodiversity and soil functioning, with specific effects for conservation and organic systems (Figure D.1).

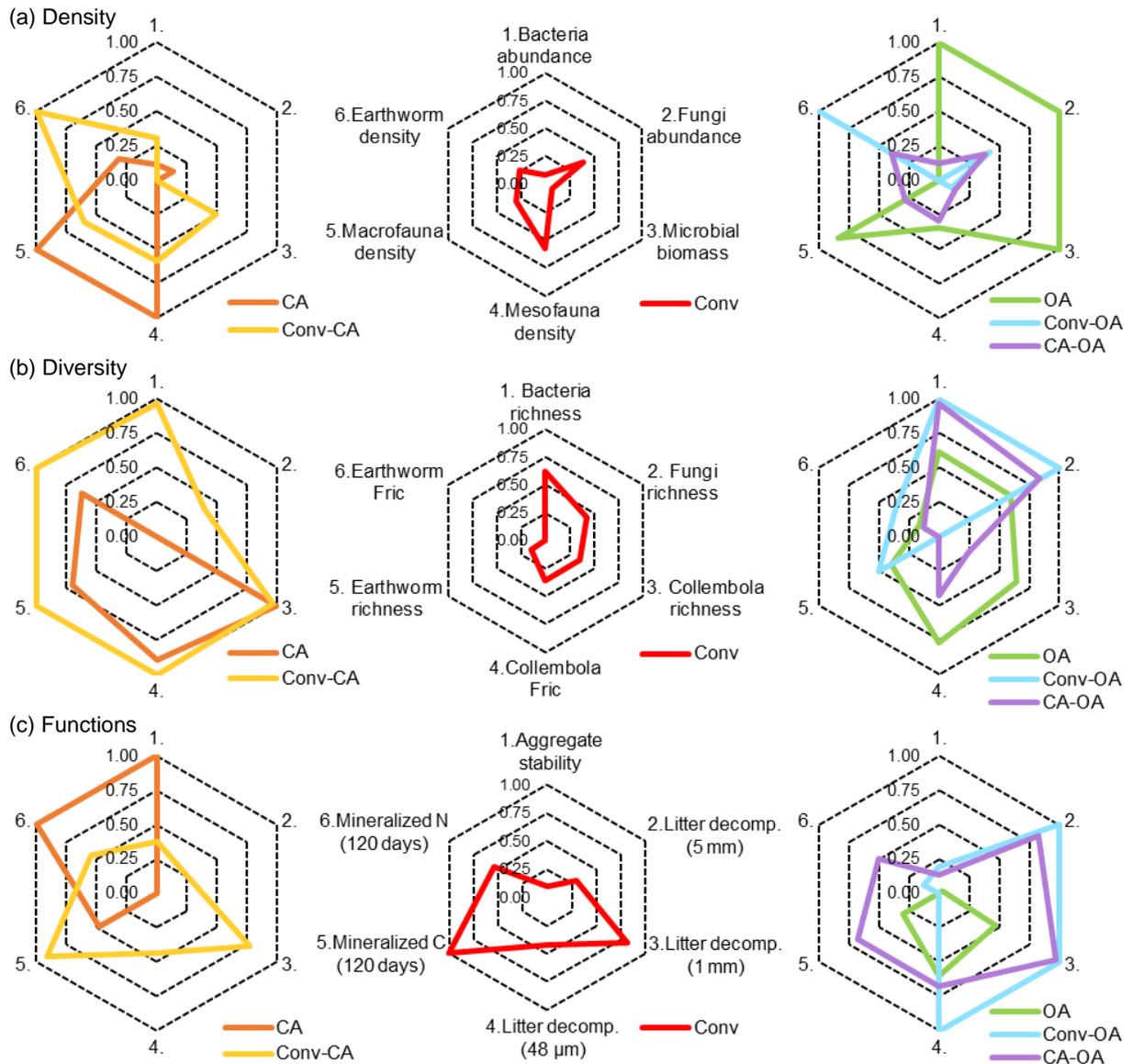


Figure D. 1 Comparative effects of conventional, conservation and organic systems on (a) density and (b) diversity of soil organisms and (c) soil functions. Stable and transitioning systems under conservation (left) and organic agriculture (right) are represented on the same plot. Values are normalized means of all plots of each system, and combine 2020 and 2021 results for density and diversity. Microbial density, diversity and biomass are averaged at 0-20 cm depth. Mineralized N and C are specific amounts of mineralized C and N after 120 days at 0-10 cm depth. Litter decomposition is the litter mass loss in litterbags of different mesh sizes after 5 months. Conv, CA and OA: conventional, conservation and organic systems; Conv-CA, Conv-OA and CA-OA: initial-recent system transitions; FRic: functional richness.

Conservation systems benefit the density and diversity of soil mesofauna and macrofauna, whereas organic systems tend to promote the density and diversity of soil microorganisms and to a lower extent of soil macrofauna (Figure D.1a and b). In addition, conservation systems significantly promote soil aggregate stability and tend to hasten N mineralization (Figure D.1c), which suggests a lower sensibility to erosion and soil crusting and increased nutrient availability for crops. However, this could also increase nitrate leaching and NH₃ emissions. In addition, conservation systems also show the highest density of Gastropoda suggesting higher risks of crop

damage due to pest species. On the other hand, organic systems have fewer and non-significant effects on soil functions despite their tendency to promote litter decomposition by microorganisms and to slow C and N mineralization (Figure D.1c). This could indicate an increase in soil organic carbon storage and thus benefits for climate regulation, but a lower nutrient availability for plants with a potential decrease in crop yield. Yet, despite differences in soil functioning, we did not observe a significant increase in SOC stocks in conservation or in organic systems compared to conventional systems.

However, the effects of cropping systems on the density and diversity of soil organisms are highly variable between years. This variability may be explained by differences in climatic conditions in both years of our study, which can affect the population dynamics of soil organisms, as well as by differences in cultivated crops. Indeed, all plots were cultivated with wheat in 2020, but with different crops in 2021 as we decided to keep the same plots. This choice relies on the hypothesis that local changes in soil properties have more effect on soil biodiversity than crop type. Indeed, we observed no significant effects of crop type on soil organisms, whereas soil microorganisms, for instance, responded to differences in soil properties. Yet, the crop diversity in 2021, and especially the fact that some plots had cover crops or no crop at all, led to a higher variability in soil cover and applied practices between plots than in 2020. Therefore, long-term studies as well as studies under various contexts are required to confirm or invalidate the trends observed in this study. More generally, studies under real conditions are valuable precisely as they encompass the existing variability within cropping systems and should be reported even if no significant differences are found. Indeed, they could be included in future reviews or meta-analyses similar to the one we conducted on litterbag experiments (Chapter 5). Thus, studies under real conditions will help to improve our understanding of the various mechanisms occurring in soils after the implementation of alternative systems.

Overall, our study tends to confirm the benefits of alternative systems on the density of soil organisms already reported on experimental sites (Henneron et al., 2015; Pelosi et al., 2015), but with observations conducted in real farms. It also shows that alternative systems have the potential to promote several soil functions and therefore ecosystems services.

How do soil organisms and soil functions respond to the transition toward alternative systems?

The conversion to alternative systems induces a period of transition during which changes in the applied practices can cause drastic changes in soil properties and in soil communities. Only few studies have been conducted on soil biodiversity and soil functioning in transitional systems, most of which focused on the conversion from conventional to organic systems (e.g. Lundgren et al., 2006; Schrader et al., 2006; Santos et al., 2012) or from conventional to no-tillage systems (e.g.

Stubbs et al., 2004; Pelosi et al., 2016). In addition, there is a lack of research on the transitions between both alternatives. In particular, we found no previous work assessing the effects on soil of the transition from conservation to organic systems. Here, we investigate soil biodiversity and soil functions in plots recently converted to alternative systems including three types of transition, namely from conventional to organic, conventional to conservation and conservation to organic agriculture. Our results show that systems in transition have important effects on the density, diversity and community structure of soil organisms (Chapters 1 to 4), and less significant effects on soil functions (Chapter 6). Most of their features differ from their initial system (i.e. conventional, conservation) and from the system they are transitioning to (i.e. organic, conservation) (Figure D.1).

Earthworm communities reportedly show transitory effects following the conversion to alternative systems (Pelosi et al., 2015, 2016). In our study, both recent organic and conservation systems indeed have a higher earthworm density than conventional and stable alternative systems (Figure D.1a), but an increase in taxonomic and functional diversity is mostly observed in recent conservation systems (Figure D.1b).

Furthermore, recent conservation systems tend to have a higher density of bacteria, lower density of fungi, and to strongly foster the diversity of bacteria, Collembola and earthworms compared to conventional and stable conservation systems (Figure D.1a and b). However, recent conservation systems tend to resemble conventional systems in terms of soil functioning except for a slight increase in aggregate stability, providing another clue on the progressive changes occurring in soils (Figure D.1c). On the other hand, recent organic systems transitioning from conventional agriculture (Conv-OA) have an overall lower density of soil organisms (except for earthworms) than conventional and stable organic systems, whereas systems transitioning from conservation to organic agriculture (CA-OA) tend to show similar densities than conventional systems (Figure D.1a and b). However, both Conv-OA and CA-OA have a higher bacterial and fungal diversity compared to conventional and stable organic systems (Figure D.1b) and tend to promote litter decomposition (Figure D.1c).

To summarize, in the first years of transition from conventional to alternative systems or from one alternative to the other, cropping systems present transitory effects on soil biodiversity and functioning. These effects may eventually result in lower or more variable yield during the transition period. However, the observed effects can vary according to the practices applied at the beginning of the transition or to the environmental variables (Schipanski et al., 2014; Jabbour et al., 2016; Gareau et al., 2019). In our study, systems in transition do not present more variability in the density or diversity of soil organisms than stable systems. However, they show some differences with stable systems, which could be related to a progressive change in disturbances and in soil properties. Furthermore, changes in soil biodiversity are observed to be even more

important during the transition from conservation to organic systems than during the transition from conventional to conservation or organic systems, surprisingly leading the former systems to resemble intensive systems. This is probably due to more intense changes in soil disturbances, switching from chemical to physical disturbances. Therefore, more studies are required on transitioning systems, and more specifically on transitions involving conservation systems, in order to fully assess the changes in soil biodiversity and time required to reach similar features to stable alternative systems.

Which agricultural practices have the largest impact on soil organisms?

In this study, we characterize cropping systems using indicators developed by agronomists. This approach has only been reported in few studies in soil ecology (Ferraro and Ghera, 2007; Masin et al., 2020) which, to our knowledge, never included several groups of soil organisms. Tillage, pesticide treatment and fertilization intensity indexes provide interesting insight on the effects of systems relying on lower soil physical and chemical disturbances. In particular, intensity indexes further explain the effect of cropping systems on soil organisms (Chapters 1 to 4) and soil functions (Chapter 6), and highlight the benefits of lower tillage, less pesticides inputs and more organic matter inputs (Figure D.2).

Tillage is considered as “the largest geo-engineering activity on Earth” (Or et al., 2021) with many observed consequences on soils. Unsurprisingly, we also show tillage intensity to be the main driver of the effect of cropping systems on soil organisms. Among other effects, lower tillage intensity benefits the density and diversity of soil mesofauna and the density of earthworms. These benefits stem directly from a decrease in physical damage on soil fauna and indirectly from an increase in microhabitats. Inversely, higher tillage intensity promotes bacterial and fungal density and diversity deeper in soils through the mixing of soil organic matter and the increasing access to trophic resource. Furthermore, tillage plays an important role in the functioning of the soil food web in cropping systems as it influences several trophic groups with cascading effects on others. Lastly, in relation with observed benefits on soil organisms, lower tillage intensity promotes higher soil structural stability and C and N mineralization, leading to an increase in soil physical and chemical fertility.

Pesticide treatments are reported to have negative effects on soil organisms, in particular on soil fauna (Pelosi et al., 2014; Joimel et al., 2022). In most systems, pesticide treatments and tillage are inversely related because they are the two means to control weeds. In our study, pesticide treatments have less important and more variable effects on soil organisms than tillage. Lower pesticide treatment intensity shows negative effects on several faunal groups, but positive effects on fungal diversity at the soil surface. These effects may however be related to the higher tillage

intensity in systems with no pesticide treatments (i.e. organic systems). In line with this, pesticides have low and non-significant effects on the soil food web. Furthermore, lower pesticide treatment intensity has the potential to promote litter decomposition and to slow C mineralization rate, which could foster soil organic carbon storage.

Organic matter inputs are often reported as a practice that benefits soil biodiversity compared to mineral fertilizers inputs (Birkhofer et al., 2008; de Souza and Freitas, 2018). In our study, the overall effects of fertilization is less important than those of other practices. Yet, an increase in the number of organic matter inputs promotes soil microbial biomass deeper in soil and influences the soil food web at different levels by increasing SOM and macrofauna detritivores. In addition, organic matter inputs are related to a decrease in C and N mineralization rate and promote SOC stock, thus being a major practice to foster climate regulation.

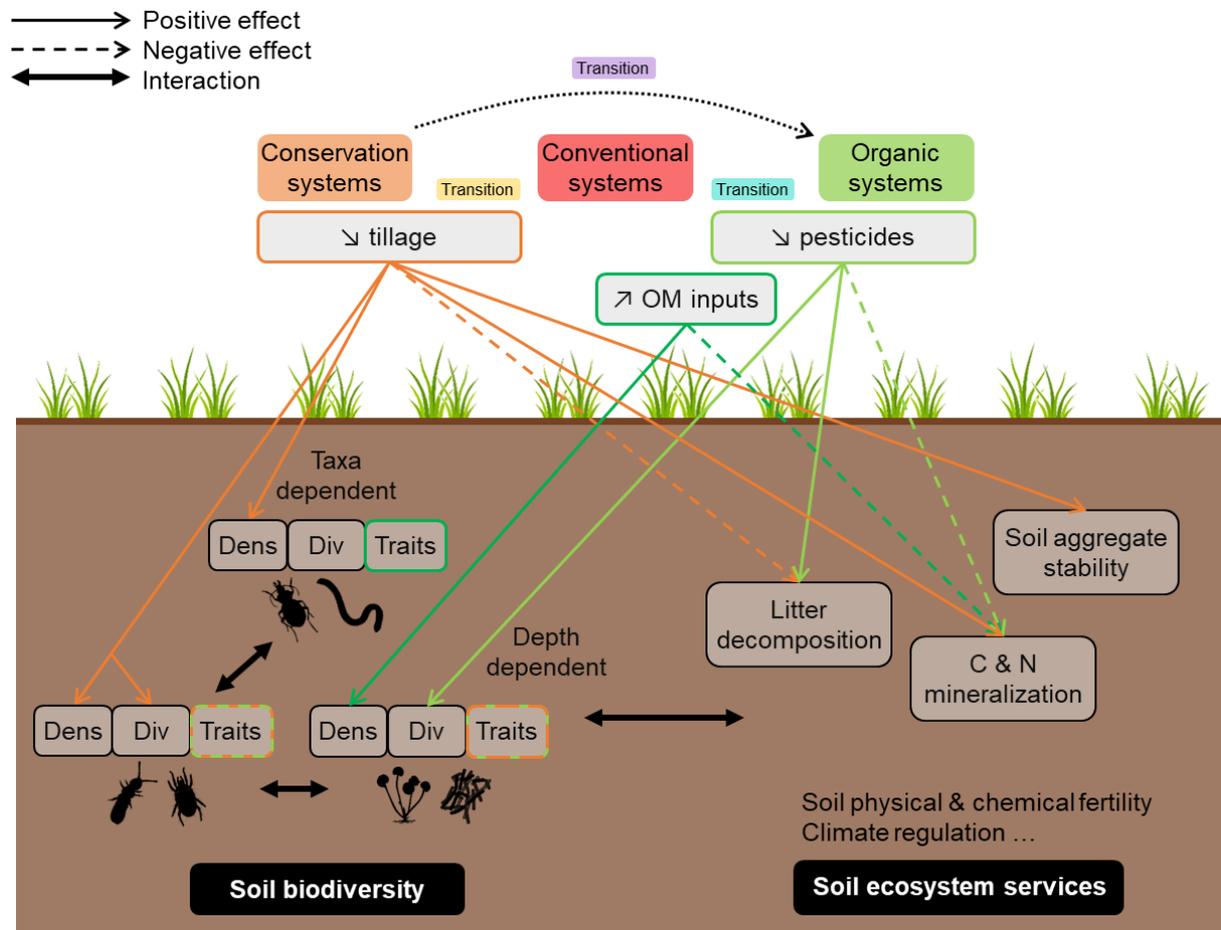


Figure D. 2 Schematic representation of the benefits of practices associated to alternative cropping systems on soil biodiversity and effects on the associated soil functions. Soil biodiversity is represented by the density and diversity of macrofauna, mesofauna and microorganisms, and by functional traits of earthworms, Collembola and bacteria. Simple arrows represent the effect of practices on soil organisms (only positive effects are represented) or on soil functions (continuous line = positive effect, dotted line = negative effect). Double-headed arrows represent interactions between soil organisms observed in the chapter 4 and between organisms and soil functions discussed in the chapters 5 and 6. Color borders of the “traits” boxes correspond to the color of practices influencing traits (orange = tillage, light green = pesticides, dark green = organic matter inputs).

Overall, all practices demonstrate effects on soil organisms and soil functions. However, whereas tillage has large and often negative effects, the effects of pesticide treatments and fertilization are limited to a few variables (Figure D.2). Furthermore, the effects of practices are not related to the size of organisms, but different groups are impacted by different practices according to their trophic regime and traits. Mesofauna representatives are the most sensitive to practice intensity, in particular mesofauna detritivores that seem to occupy a central place in the food web of agricultural soils. Overall, the decrease in tillage intensity in conservation systems and both the absence of pesticide treatments and higher organic matter inputs in organic systems may promote soil biodiversity and the provision of ecosystem services (Figure D.2).

Finally, which cropping systems should be developed to promote soil biodiversity?

Most of our results show that conservation agriculture has the highest benefits on soil biodiversity and its activity in soil, which is directly related to a low tillage intensity. However, organic agriculture promotes soil biodiversity as well thanks to the absence of pesticides and to more organic matter inputs, but through smaller and more widespread effects on soil communities. Overall, both organic and conservation systems provide an alternative to conventional agriculture with benefits and drawbacks on different groups of soil organisms and on the associated soil functions.

Other alternative systems relying on the benefit of different practices could be developed in upcoming years. For instance, an increasing number of studies are conducted on organic systems combined with lower tillage intensity (Peigné et al., 2007, 2009, 2018; Zikeli and Gruber, 2017; Boeraeve et al., 2022; Krauss et al., 2022). While these hybrid systems are promising, previous studies report inconsistent effects on soil properties and soil organisms, and advocate for more research and for the development of technical advice before larger implementation. Besides, there are endless possibilities of combinations between practices and environmental contexts, which may for instance drive the selection of cropping systems or practices that are more adapted to local environmental risks (i.e. erosion, risk of water pollution).

This study emphasizes the importance of taking into account soil biodiversity and soil functioning to assess the sustainability of cropping systems. However, observations on the belowground and aboveground compartments should be combined in order to have a comprehensive overview of the effect of cropping systems on biodiversity and ecosystem services. In addition, the development or selection of alternative systems should necessarily take into account the trade-offs between the promotion of biodiversity and environmental benefits, and crop production to ensure that systems are sustainable.

More generally, this study illustrates that there is not a unique solution to increase agricultural sustainability. To avoid repeating the mistakes of the past green revolution, several alternative systems should be promoted rather than a unique system that may be seen as the “best” solution. Overall, to promote soil biodiversity, as well as the sustainability of crop production, the optimal solution is probably to promote cropping system diversity.

Which approaches can best characterize the effects of cropping systems on soil biodiversity and soil functioning? – A feedback on methodological choices

Are indicators of practice intensity developed by agronomist relevant for soil ecologists?

Indicators developed by agronomists seem to provide a useful mean to determine more precisely the effects of cropping systems on soil organisms. They show significant and large effects on soil organisms (Chapters 1 to 4) and on soil functions (Chapter 6) and unveil effects that were hidden with the usual cropping system approach.

In this thesis, we use the additive aggregation method to combine primary indicators and compute composite indexes on tillage, pesticide treatments and fertilization following Büchi et al. (2019). Composite indexes representing tillage and pesticide treatment intensities demonstrate numerous and highly significant relations with the density and diversity of soil organisms and soil functions. In contrast, the fertilization intensity index shows few significant effects on soil biodiversity. This result can partly be explained by the construction of the index. To construct the fertilization index, we considered that all practices included in the index, namely organic matter inputs, mineral N inputs and crop residues exportations, conduct to an increase in the fertilization intensity, as presented in Büchi et al. (2019). We selected this method in order to have a similar representation of practice intensity than for tillage and pesticide treatments (i.e. an overall score of disturbances related to fertilization). Alternative solutions would be to determine a positive or negative effect of the different practices or to use PCA scores. However, both of these methods oppose organic *versus* mineral fertilization rather than showing the intensity of fertilization practices. In addition, attributing a positive or negative value to organic or mineral inputs and to residues exportations is complex as they provide positive or negative effects depending on groups of soil organisms, and complex relation to plant growth.

Regarding primary indicators, we show that the number of organic matter inputs influences several organisms and soil functions without requiring much details on the applied product (i.e. type or dose), hence being a relevant and easily accessible indicator. Therefore, future studies could develop a more exhaustive index on organic matter fertilization based on easily accessible information. For instance, such index could account for the stability of organic matter (i.e.

composted or not) and for the time between application and sampling. A second step could be to determine an average score for the most common inputs (e.g. manure, pellets, green wastes) depending on their C and N content, and to calculate a mean score for organic matter inputs occurring during the years preceding the sampling.

Is the functional trait approach relevant to study soil organisms in cropping systems?

Functional traits are increasingly used in soil ecology. Yet, to our knowledge, no study conducted a multi-organisms approach of traits in agricultural soils. In this thesis, traits are assessed for earthworms, Collembola and bacteria (Chapters 1 to 3) in an effort to provide a better understanding of the factors influencing the functional diversity of soil organisms in cropping systems.

We show that traits provide few but non-negligible additional information on the effects of disturbances caused by practices on Collembola and on earthworms. For earthworms, traits however reveal similar effects than species owing to the very low number of species in agricultural soils. Furthermore, many bacterial functional traits are influenced by cropping systems and practice intensity, especially by tillage, but with a high variability between years. Yet, despite this variability, several traits show a similar response to cropping systems, tillage and pesticide treatment intensities in both years. These traits could be relevant to better understand the selection occurring in bacterial communities in different cropping systems. However, larger samplings are required to recommend a set of bacterial traits that are reliably representative of the effects of disturbances occurring in agricultural areas. In addition, trait information are missing for a large number of bacterial strains and more data would be required to calculate indexes of functional diversity. More generally, it is currently difficult to compare results of functional traits for soil invertebrates and microorganisms as they rely on different approaches.

The measurement of length traits on collected Collembola allows to assess intra-specific variability and appears to be more relevant than using average length traits of species to assess the effect of practices on the soil fauna. Therefore, even if trait measurement is time consuming, it is worthy of more investigations in agroecosystems. In addition, we found similar results for all Collembola length traits, suggesting that measurement of body length may be sufficient to assess the effect of disturbances on Collembola. However, this assumption should be tested under different contexts, as well as for other groups of soil organisms. For each group of soil organisms, specific length traits (e.g. furca for Collembola, elytra for ground beetle) or other traits (e.g. hairiness, color) could be relevant measurements (Pey et al., 2014; Moretti et al., 2017). More generally, measuring body length on other groups of soil organisms (e.g. Acari, Earthworms,

Carabidae) could reveal whether disturbances cause an overall change in the size of soil organisms or if their effects on the soil community are more complex.

Overall, functional diversity based on functional traits is promising to further our understanding of the effect of cropping systems and associated disturbances on soil organisms. The assessment of functional traits may be especially relevant for groups with a high number of species and requiring precise identification skills.

D.2 Perspectives

D.2.1 Assessing the effects of cropping systems on soil organisms in different seasons

In temperate areas, the density of soil organisms is the highest in spring and in autumn due to better moisture and temperature conditions in soil. Our study was conducted in autumn, right after wheat sowing, a period at which the effect of physical disturbances caused by tillage and sowing are strong. However, the effects of cropping systems on soil organisms could be different at other times of the year.

The timing of both tillage implementation and sampling of soil organisms could be a main factor explaining variability between studies (Kladivko, 2001). Indeed, soil species were reported to recover after tillage at more or less long terms (Schmidt and Curry, 2001; Wortmann et al., 2008; Pelosi et al., 2016). Therefore, the effects of tillage and of other practices could be perceived differently at different steps of the crop cycle. In addition, the sensitivity of different groups of soil organisms varies throughout the year. For instance, invertebrates are more sensitive to tillage during periods where the density of eggs, larvae or juveniles is the highest, and inversely can be less impacted during their overwintering period (Roger-Estrade et al., 2010). However, few studies compare the effect of different cropping systems in two seasons. Previous studies reported higher earthworm abundance in no-till than in conventional systems in spring, while no differences were observed in autumn (Schmidt and Curry, 2001; Reeleder et al., 2006). In contrast, few differences in microarthropod abundance or diversity were observed between spring and autumn in conventional and no-tillage fields (Arroyo and Iturrondobeitia, 2006; Brennan et al., 2006).

As we observed major negative effects of tillage on mesofauna in autumn and as mesofauna detritivores seem to have a central place in the soil food web of agricultural soils, it would be interesting to also assess mesofauna density and diversity in spring. To this aim, mesofauna was sampled in the 21 plots of this study in spring 2021 and 2022 and future investigations may determine if a seasonal effect is observed.

D.2.2 Estimating the biodiversity-function relations in cropping systems

In the last decades, an increasing number of studies were conducted on the links between biodiversity and ecosystem functions by developing biodiversity-ecosystem functioning (BEF) experiments. They show that functional diversity is more relevant than species richness to study the effect of biodiversity on ecosystem functioning (Heemsbergen et al., 2004; Reiss et al., 2009; Coulis et al., 2015). The association between functional traits and ecosystem services was reported for plant communities and to a lesser extent for soil organisms (de Bello et al., 2010).

In our study, we measured separately soil biodiversity and soil functions in cropping systems. Therefore, the links between biodiversity and functions had to be discussed based on the literature. However, our study brings additional information on the organisms usually encountered in agricultural soils and on the diversity occurring in different cropping systems. This could help construct more realistic BEF experiments, for which the accurate representation of selected soil organisms and the level of diversity are main limits. To go further in that direction, a mesocosm experiment could be conducted with the aim of measuring soil functions (i.e. litter decomposition, stabilization of the soil structure) under different combinations of soil organisms representing communities of different cropping systems. These combinations would unavoidably be simplistic models representing major differences observed between communities. They could for instance replicate changes in the soil food web (i.e. density or diversity of trophic groups) between different cropping systems. Another option would be to determine combinations that include similar trophic groups but with changes in their functional diversity, such as by removing smaller or larger organisms as suggested by Reiss et al. (2009). However, in this case, the determination of combinations representing different cropping systems will require the conduction of additional trait measurements on the organisms collected during field sampling.

D.2.3 Determining soil biodiversity in agricultural areas at the landscape scale

An increase in management heterogeneity within agricultural landscapes was reported to promote biodiversity and the associated services (Purtauf et al., 2005; Diekötter et al., 2010). However, while the landscape approach was widely developed for aboveground organisms, few studies have focused on belowground organisms that are less mobile.

The environment of sampled fields could be characterized in order to add a new dimension to our observations on soil biodiversity in agroecosystems. This involves an assessment of the landscape at a small scale around sampled fields. A similar approach was used recently to evaluate pest regulation around several fields belonging to the network of farmers on which our study is based (Serée et al., 2022). It could be applied to assess soil biodiversity by taking into account the

semi-natural areas, as in the previously mentioned study, and by assessing the range of physical and chemical disturbances in the surroundings of the fields. This could be achieved by estimating a percent of areas that are undisturbed (i.e. semi-natural), tilled and subjected to pesticide treatments. More generally, novel studies using landscape ecology and accounting for soil biodiversity could investigate if landscapes composed of a diversity of cropping systems provide an overall greater density and diversity of soil organisms than landscapes managed under a single alternative system.

D.3 Shedding light on soil biodiversity: from research to science outreach

This part constitutes an opening on the role of researchers in science popularization or science outreach. It comprises a feedback on the mission I had the opportunity to conduct in a science museum, the “Cité des Sciences et de l’Industrie” in Paris, during the last year of this PhD project. This mission included the development and the animation of a workshop on soil and its biodiversity for the general public.

The involvement of researchers in science popularization is important as they bring scientific content and are generally aware of popular misconceptions. Moreover, working with specialists such as scientific mediators is valuable for scientists because they hold keys for efficiently sharing ideas with a diverse audience. In particular, they can identify words that are part of the scientific jargon and help to find appropriate translations. They also suggest different media (e.g. science show, games, workshops) adapted to the nature of the scientific content (Figure D.3).



Figure D. 3 A stand on soil biodiversity during the Science Infuse event at the Cité des Sciences et de l’Industrie (left) and the Biolab (popular science lab) with material for a workshop on soil structure (right).

There is currently a growing interest for soils. Researchers and in particular young scientists definitely have their role to play in the public awareness on soil life. Bringing attention toward soil biodiversity is an essential step to further the understanding of the importance of soils and to promote the consideration of soil organisms in conservation policies. Indeed, soil organisms are the “unseen” part of the biodiversity. They are often unloved or disregarded due to a large unfamiliarity and a long association of soil to dirt, but more importantly, some people simply never heard about them. In large cities, as in Paris, most people have a limited or no access to uncovered soil. However, even for people with gardens or living in rural areas soil organisms are mostly considered as pests, except maybe for earthworms.

Composts are a good entry door to present soil organisms and to explain soil processes. They are now accessible in many places even in cities and a large number of people have their own compost at home. It is for instance a nice place to find organisms of different sizes and shapes such

as Collembola (i.e. those little running white dots), earthworms, larvae or myriapods. A focus on mesofauna can be particularly interesting. Indeed, Collembola and Acari are largely unknown, which gives them the potential to arouse the curiosity of people, and their size between the invisible microorganisms and the large insects provides an opportunity to discuss about the different size groups of soil organisms. In addition, they are a good case study for observations with binoculars and microscopes. These observations open discussions on the role of organisms in soil according to their morphological aspects (or in other words their traits!) and definitely shed the light on the unsuspected biodiversity of soils.

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———— Appendices ————

Appendix 1 - Supplementary material Chapter 1

Appendix 1.1 Collembola species frequently observed in our study and their relation to cropping systems and practice intensity

Pseudosinella alba (Packard, 1873) and *Isotomurus palustris* (Muller, 1776) species were collected in all systems in both years (Table S1.4). Other species collected in all systems were *Stenacidia violacea* (Reuter, 1881) in 2020, and *Entomobrya multifasciata* (Tullberg, 1871), *Isotoma viridis* Bourlet, 1839, *Lepidocyrtus cyaneus* Tullberg, 1871, *Orchesella villosa* (Geoffroy, 1762) and *Parisotoma notabilis* (Schaeffer, 1896) in 2021 (Table S1.4). Two species were collected exclusively in 2020 against 15 species in 2021, including 12 species occurring in one plot only. More precisely, we found no *O. villosa* in 2020 whereas this species was collected in all systems in 2021. In addition, some species were absent from all organic plots in both years such as *Desoria violacea* (Tullberg, 1877), *Folsomia candida* V.Willem, 1902 and *Sminthurinus aureus* (Lubbock, 1862), or less frequent as for *Brachystomella parvula* (Schäffer, 1896), *Protaphorura armata* Lawrence, 1979, *Lepidocyrtus lanuginosus* (Linnaeus, 1788) or *Lepidocyrtus lignorum* (Fabricius, 1793) (Table S1.4). In conventional system, *Isotoma anglicana* J.Lubbock, 1862 and *Sminthurides parvulus* (Krausbauer, 1898) were absent (Table S1.4).

Cryptopygus thermophilus (Axelson, 1900) and *Sminthurinus elegans* (Fitch, 1863) densities were significantly higher in Conv-CA than in other systems in 2020 and in CA than in Conv and CA-OA in 2021 (GLM, $P < 0.05$). In addition, in 2020, *L. cyaneus* density was higher in Conv than in Conv-OA, and *P. notabilis* density was higher in Conv than in Conv-CA and Conv-OA. In 2021, *I. viridis* density was significantly higher in CA than in other systems except Conv-CA, and *Mesaphorura macrochaeta* Rusek, 1976 was higher in Conv-CA than in Conv, Conv-OA and CA-OA.

Densities of *L. cyaneus* and *P. alba* were lower under higher tillage intensity in both years (Table S1.5), and similar effect was observed for *C. thermophilus*, *I. palustris* and *M. macrochaeta* in 2020, and *I. viridis* and *S. elegans* in 2021 (Table S1.5). Inversely, higher pesticide treatment intensity was related with higher densities of *L. cyaneus* and *P. alba* in both years and of *P. armata* and *S. elegans* in 2020 only. Higher fertilization intensity was related to a decrease in the density of *I. palustris* density in 2020 and *I. viridis* in 2021 (Table S1.5).

Figure S1.1 Principal coordinate analysis of Collembola species community (a) in 2020 and 2021 (ANOSIM system: $R = 0.135, P = 0.016$; ANOSIM year: $R = 0.130, P = 0.005$), and in the different cropping systems (b) in 2020 (ANOSIM system: $R = 0.241, P = 0.021$) and (c) in 2021 (ANOSIM system: $R = 0.078, P = 0.231$). Conv: conventional agriculture; CA: conservation agriculture; OA: organic agriculture; Conv-CA, Conv-OA and CA-OA: initial-recent system transitions.

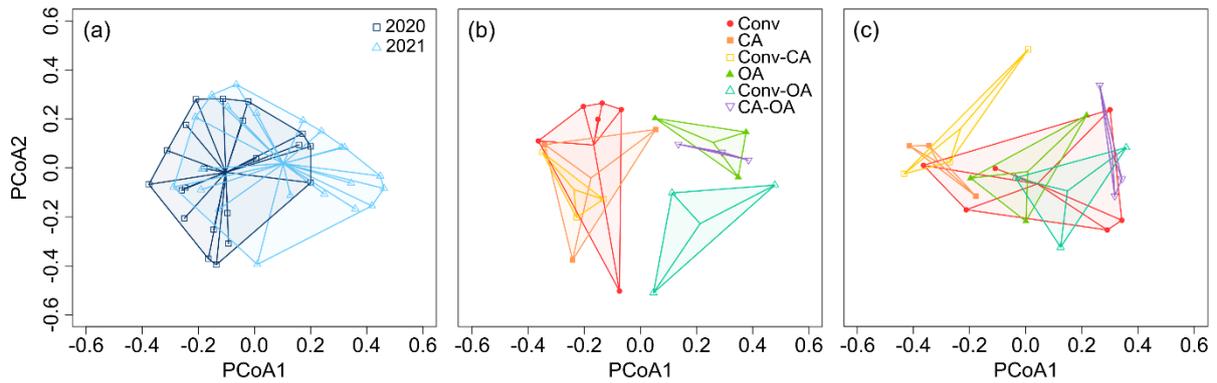


Figure S1.2 PCA on community-weighted mean of Collembola traits in (a) 2020 and (b) 2021. Conv, CA and OA: conventional, conservation and organic agriculture; Conv-CA, Conv-OA and CA-OA: initial-recent system transitions.

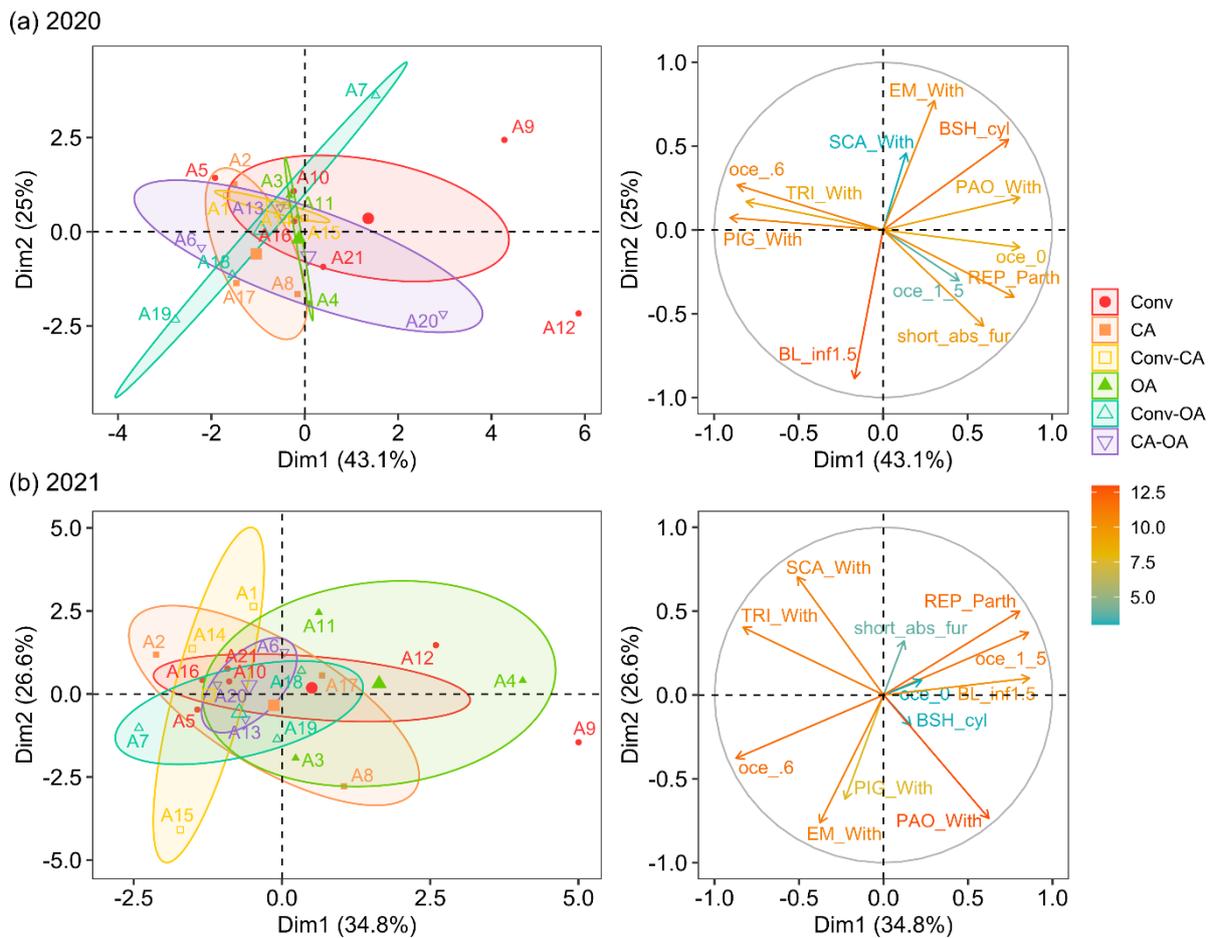


Figure S1.3 Effect of (a) tillage, (b) pesticide treatment and (c) fertilization intensity on Collembola total body length in 2020 (circles, dark/purple line) and 2021 (triangles, light/green line). Thick line results from GLM with a negative binomial distribution. Colored areas are the 95% confidence interval.

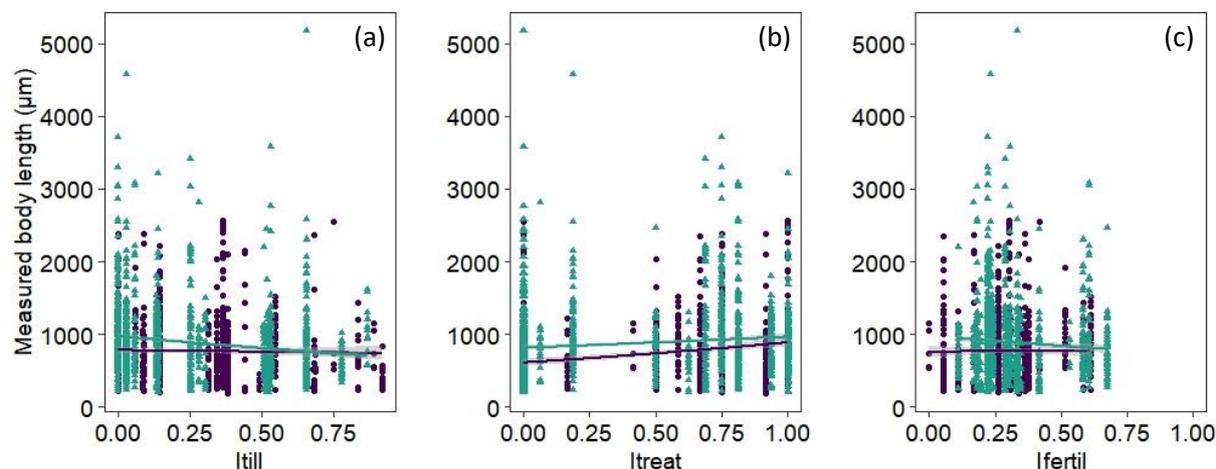


Table S1.1 Effect of interactions of intensity indexes on soil mesofauna in (a) 2020 and (b) 2021. *t*-value and *P*-value were obtained using GLM with a quasi-Poisson error distribution. Bold values indicate significant effects ($P < 0.05$ *, < 0.01 **, < 0.001 ***).

	Itill		Itreat		Ifertil		Itill*Itreat		Itill*Ifertil		Itreat*Ifertil		Itill*Itreat*Ifertil	
	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>
(a) 2020														
Total mesofauna	0.06	0.954	1.13	0.277	0.21	0.841	-0.97	0.349	-0.22	0.828	-0.86	0.406	1.54	0.147
Acari	0.29	0.778	1.14	0.274	0.47	0.646	-1.03	0.32	-0.48	0.637	-0.99	0.339	1.68	0.117
Actinidida	-0.42	0.684	0.48	0.641	-0.74	0.474	-0.55	0.591	0.56	0.582	-0.08	0.938	0.27	0.789
Gamasida	-1.51	0.156	-0.33	0.746	-1.31	0.214	0.40	0.693	1.28	0.222	0.82	0.426	-0.02	0.983
Oribatida	0.57	0.578	1.32	0.211	0.71	0.488	-1.19	0.257	-0.72	0.482	-1.17	0.264	1.84	0.089
Collembola	-1.21	0.247	1.12	0.283	-1.41	0.181	-0.59	0.565	1.34	0.202	0.01	0.989	0.26	0.799
Epedaphic	-1.03	0.323	1.14	0.273	-1.17	0.262	-0.75	0.466	1.08	0.299	-0.24	0.816	0.21	0.838
Euedaphic	-0.87	0.399	-1.36	0.196	-1.15	0.272	1.31	0.212	0.94	0.362	1.49	0.16	0.19	0.854
Hemiedaphic	-0.33	0.748	1.53	0.15	-0.46	0.657	-1.02	0.326	0.59	0.564	-0.29	0.778	0.38	0.71
Other	-0.45	0.657	1.31	0.214	-1.04	0.317	-0.67	0.516	0.91	0.378	-0.62	0.544	1.79	0.097
Collembola richness	-2.58	0.023*	-0.30	0.767	-3.48	0.004**	0.31	0.761	3.11	0.008**	2.17	0.049*	-1.26	0.231
(b) 2021														
Total mesofauna	-0.20	0.842	0.75	0.468	0.56	0.582	-0.04	0.972	-0.31	0.76	-0.88	0.396	-0.07	0.948
Acari	0.32	0.752	0.66	0.522	0.60	0.561	-0.27	0.789	-0.55	0.593	-0.67	0.512	0.02	0.984
Actinidida	0.03	0.98	0.29	0.78	-0.40	0.698	0.34	0.738	-0.20	0.847	-0.41	0.689	-0.27	0.791
Gamasida	-0.68	0.506	-0.83	0.423	-0.73	0.477	0.86	0.406	0.63	0.543	0.62	0.546	-0.95	0.359
Oribatida	0.46	0.656	0.87	0.403	0.76	0.463	-0.48	0.64	-0.68	0.511	-0.84	0.416	0.18	0.862
Collembola	-0.31	0.76	0.92	0.374	0.60	0.562	-0.13	0.898	-0.30	0.77	-1.06	0.311	0.16	0.873
Epedaphic	-0.25	0.804	1.57	0.142	0.62	0.546	-0.68	0.508	-0.39	0.703	-1.17	0.263	0.23	0.821
Euedaphic	0.32	0.757	0.84	0.417	0.59	0.566	-0.16	0.877	-0.16	0.873	-0.73	0.481	-0.11	0.912
Hemiedaphic	-0.52	0.612	0.39	0.706	0.36	0.725	0.27	0.789	-0.04	0.972	-0.77	0.455	-0.07	0.948
Other	-0.70	0.494	0.50	0.628	-0.44	0.671	0.63	0.542	0.80	0.44	-0.10	0.921	-1.06	0.308
Collembola richness	-0.10	0.921	1.73	0.108	1.44	0.175	-0.12	0.91	-0.47	0.647	-1.72	0.11	-0.41	0.691

Itill: tillage intensity, Itreat: pesticide treatment intensity, Ifertil: fertilization intensity.

Table S1.2 Density, richness and diversity indexes of Collembola in the 21 plots of the study in 2020 and 2021.

Plot	2020					2021												
	Density			Ratio A/C	Collembola diversity					Density			Ratio A/C	Collembola diversity				
	Total	Acari	Collembola		R	H	E	FRic	FEve	Total	Acari	Collembola		R	H	E	FRic	FEve
A1	34511	19624	14210	1.4	12	2.11	0.85	0.31	0.52	59142	24090	33834	0.7	15	1.88	0.69	0.36	0.33
A2	41819	32751	8662	3.8	13	2.22	0.87	0.24	0.55	68074	24225	43308	0.6	14	1.73	0.65	0.24	0.42
A3	4466	3113	1218	2.6	6	1.68	0.94	0.24	0.79	6361	2030	4060	0.5	10	2.11	0.92	0.36	0.68
A4	4466	2301	1895	1.2	5	1.38	0.85	0.13	0.58	79172	50480	27879	1.8	12	1.53	0.62	0.26	0.48
A5	99472	91081	7850	11.6	9	1.67	0.76	0.21	0.56	10556	7985	2301	3.5	5	1.43	0.89	0.14	0.58
A6	10962	7714	2977	2.6	7	1.67	0.86	0.28	0.54	14616	12180	2436	5	7	1.74	0.89	0.12	0.54
A7	2707	2030	541	3.8	3	1.04	0.95	0.05	0.56	14887	13263	1624	8.2	7	1.79	0.92	0.13	0.72
A8	15834	13398	2301	5.8	4	1.19	0.86	0.15	0.86	62661	19353	42766	0.5	12	1.76	0.71	0.28	0.36
A9	1895	947	812	1.2	3	0.87	0.79	0.01	0.55	10692	5684	4466	1.3	4	0.73	0.53	0.02	0.50
A10	32345	20300	11639	1.7	12	2.10	0.85	0.32	0.49	56706	33157	21789	1.5	11	1.81	0.76	0.34	0.61
A11	10150	7037	2842	2.5	6	1.58	0.88	0.14	0.66	26661	17188	8662	2	18	2.48	0.86	0.35	0.58
A12	38977	34240	4060	8.4	7	1.57	0.81	0.19	0.72	4195	3248	947	3.4	4	1.35	0.98	0.11	0.77
A13	10150	5819	4195	1.4	8	1.84	0.88	0.23	0.51	27203	23413	3113	7.5	9	1.99	0.91	0.19	0.61
A14	6902	4195	2707	1.6	8	1.80	0.87	0.20	0.71	45067	31669	13263	2.4	13	1.61	0.63	0.38	0.60
A15	10421	4737	5278	0.9	11	1.81	0.76	0.28	0.52	22466	14752	7308	2	9	1.53	0.70	0.18	0.60
A16	25714	20571	3654	5.6	8	1.87	0.90	0.21	0.67	37623	23955	13669	1.8	14	2.08	0.79	0.27	0.57
A17	33022	23413	9338	2.5	12	2.02	0.81	0.34	0.67	38706	25849	12045	2.1	14	2.13	0.81	0.34	0.50
A18	3519	3248	271	12.0	2	0.69	1.00	NA	NA	22060	16511	5278	3.1	10	1.92	0.84	0.20	0.49
A19	4601	3383	1218	2.8	3	0.96	0.88	NA	NA	14887	4060	10150	0.4	10	1.76	0.76	0.23	0.52
A20	14210	13398	677	19.8	4	1.33	0.96	0.11	0.94	43578	40872	2707	15.1	8	1.64	0.79	0.22	0.70
A21	23549	18812	4195	4.5	9	2.09	0.95	0.16	0.69	11910	9068	2842	3.2	8	1.72	0.83	0.17	0.65

Ratio A/C: ratio of Acari/Collembola density, R: species richness, H: Shannon index, E: Pielou's evenness index, FRic: functional richness, FEve: functional evenness.

Table S1.3 Mean density (ind.m⁻²) of Collembola species collected in different cropping systems in 2020 and 2021. Only species collected at least in two plots in 2020 or 2021 are presented. Figures in bold correspond to the most frequent species in terms of the number of occurrence in each system.

Species	Conv		CA		Conv-CA		OA		Conv-OA		CA-OA	
	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
	<i>Brachystomella parvula</i>	90	0	90	45	45	0	0	0	0	0	135
<i>Cryptopygus thermophilus</i>	23	180	361	4917	1173	947	0	541	45	0	0	0
<i>Desoria violacea</i>	23	113	45	45	0	0	0	0	0	0	0	0
<i>Entomobrya lanuginosa</i>	90	203	316	0	496	0	0	496	0	135	0	0
<i>Entomobrya multifasciata</i>	406	23	90	180	226	180	0	180	0	135	180	90
<i>Folsomia candida</i>	90	0	0	0	0	90	0	0	0	0	0	0
<i>Isotoma anglicana</i>	0	0	0	0	0	451	45	45	0	0	0	0
<i>Isotoma viridis</i>	68	361	45	4421	45	722	0	316	45	406	45	90
<i>Isotomurus palustris</i>	361	383	180	541	1218	1308	90	180	90	135	541	180
<i>Lepidocyrtus cyaneus</i>	1218	2729	992	9880	1083	6271	271	1038	0	1353	180	496
<i>Lepidocyrtus lanuginosus</i>	0	0	0	1353	0	2165	0	0	0	45	0	0
<i>Lepidocyrtus lignorum</i>	23	90	135	677	45	0	45	406	0	0	0	0
<i>Megalothorax minimus</i>	135	203	0	45	0	90	0	90	0	45	180	0
<i>Mesaphorura macrochaeta</i>	68	0	271	45	226	180	0	135	45	0	90	0
<i>Orchesella villosa</i>	0	68	0	361	0	226	0	180	0	90	0	90
<i>Parisetoma notabilis</i>	451	1624	361	3158	0	1985	316	6045	0	722	135	361
<i>Proisetoma minuta</i>	90	293	0	4331	0	0	361	180	0	1398	0	271
<i>Protaphorura armata</i>	541	68	45	45	45	406	0	992	0	0	0	0
<i>Pseudosinella alba</i>	406	293	632	1940	90	2030	90	586	45	90	45	226
<i>Pseudosinella immaculata</i>	0	23	0	180	0	0	0	135	0	135	0	0
<i>Sminthurides malmgreni</i>	316	90	722	0	451	90	45	451	0	316	0	45
<i>Sminthurides parvulus</i>	0	0	0	0	0	45	0	0	0	0	45	451
<i>Sminthurides schoetti</i>	203	0	0	90	0	90	0	0	0	90	0	135
<i>Sminthurides signatus</i>	429	564	632	0	0	45	0	632	226	90	361	180
<i>Sminthurinus aureus</i>	23	23	135	0	632	0	0	0	0	0	0	0
<i>Sminthurinus elegans</i>	45	0	316	316	1128	226	0	90	0	45	0	0
<i>Sphaeridia pumilis</i>	180	0	316	0	226	90	45	0	0	0	0	45
<i>Stenacidia violacea</i>	90	226	1083	90	90	0	541	90	180	226	632	90

Conv: conventional agriculture, CA: conservation agriculture, OA: organic sy agriculture stem, Conv-CA: transition from conventional to conservation, Conv-OA: transition from conventional to organic, CA-OA: transition from conservation to organic.

Table S1.4 Effect of intensity indexes on Collembola species density in 2020 and 2021. *t*-value and *P*-value were obtained using GLM with a quasi-Poisson error distribution. Bold values indicate significant effects (*P* < 0.05 *, < 0.01 **, < 0.001 ***). Results are only reported for Collembola species among the most frequent species and that were significantly related to one of the indexes.

	2020						2021					
	Itill		Itreat		Ifertil		Itill		Itreat		Ifertil	
	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>
<i>C. thermophilus</i>	-2.17	0.043*	2.03	0.056	0.61	0.547	-2.02	0.058	-0.88	0.391	-0.30	0.765
<i>I. palustris</i>	-2.96	0.008**	1.38	0.182	-2.29	0.018*	-1.70	0.105	1.73	0.101	0.64	0.53
<i>I. viridis</i>	-0.55	0.589	1.18	0.254	0.74	0.471	-2.21	0.040*	0.39	0.699	-2.47	0.023*
<i>L. cyaneus</i>	-3.04	0.007**	3.55	0.002**	-0.42	0.681	-3.32	0.004**	2.56	0.019*	-0.69	0.497
<i>M. macrochaeta</i>	-2.99	0.007**	1.15	0.265	0.90	0.378	-0.46	0.652	0.54	0.593	0.18	0.857
<i>P. alba</i>	-2.73	0.013*	3.39	0.003**	0.53	0.604	-2.83	0.011*	1.97	0.064	-1.10	0.284
<i>P. armata</i>	-0.24	0.815	2.14	0.046*	1.91	0.072	0.50	0.624	-0.13	0.902	-1.50	0.149
<i>S. elegans</i>	-0.68	0.508	2.31	0.033*	0.44	0.667	-2.91	0.009**	1.55	0.137	0.18	0.861

Itill: tillage intensity, Itreat: pesticide treatment intensity, Ifertil: fertilization intensity.

Table S1.5 Mean values of Collembola functional evenness, community-weighted mean of species traits and of measured individual traits (in μm) in the studied cropping systems. Different lower case letters and bold values indicate significant differences (*P* < 0.05) between cropping systems according to the results of GLMM with a negative binomial error distribution for measured traits and GLM with a quasi-Poisson error distribution for the other variables.

	2020						2021					
	Conv	CA	Conv-CA	OA	Conv-OA	CA-OA	Conv	CA	Conv-CA	OA	Conv-OA	CA-OA
FEve	0.6 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.6 ± 0.0	0.7 ± 0.2	0.1 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
BSH_cyl	0.8 ± 0.2	0.5 ± 0.2	0.7 ± 0.1	0.7 ± 0.1	0.5 ± 0.4	0.6 ± 0.2	0.9 ± 0.1	1.0 ± 0.0	1.0 ± 0.0	0.9 ± 0.0	0.8 ± 0.2	0.6 ± 0.2
Oce_0	0.3 ± 0.3	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.03 ± 0.04	0.01 ± 0.01	0.03 ± 0.02	0.15 ± 0.01	0.05 ± 0.05	0
M_BL	804 ± 121	637 ± 106	852 ± 38	719 ± 225	789 ± 541	731 ± 112	813 ± 91	929 ± 82	981 ± 128	819 ± 89	851 ± 218	753 ± 94
M_HL	197 ± 26	183 ± 22	215 ± 29	194 ± 41	218 ± 84	199 ± 38	187 ± 25	205 ± 16	220 ± 38	188 ± 20	202 ± 34	195 ± 6
M_LL	306 ± 71	268 ± 59	328 ± 53	284 ± 86	320 ± 192	299 ± 70	329 ± 63	366 ± 61	388 ± 48	319 ± 48	355 ± 80	337 ± 31
M_AL	252 ± 61	232 ± 44	294 ± 59	254 ± 79	290 ± 168	264 ± 66	259 ± 49	294 ± 34	312 ± 80	265 ± 34	291 ± 69	285 ± 14
M_FL	257 ± 81	236 ± 59	300 ± 44	282 ± 93	306 ± 219	274 ± 62	309 ± 51	348 ± 49	365 ± 71	293 ± 46	335 ± 83	312 ± 31

Conv: conventional agriculture, CA: conservation agriculture, OA: organic agriculture, Conv-CA, Conv-OA, CA-OA: initial-recent system transitions, BSH_cyl: cylindrical body shape, Oce_0: absence of ocelli, M_BL: measured body length, M_HL: measured head length, M_LL: measured leg length, M_AL: measured antenna length, M_FL: measured furca length.

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Figure S2.1. Principal coordinate analysis of earthworm species community (a) in 2020 and 2021 (ANOSIM year: $R = 0.008, P = 0.306$), and in the different cropping systems (b) in 2020 (ANOSIM system: $R = -0.093, P = 0.786$) and (c) in 2021 (ANOSIM system: $R = -0.094, P = 0.799$). Conv, CA and OA: conventional, conservation and organic agriculture; Conv-CA, Conv-OA and CA-OA: initial-recent system transitions.

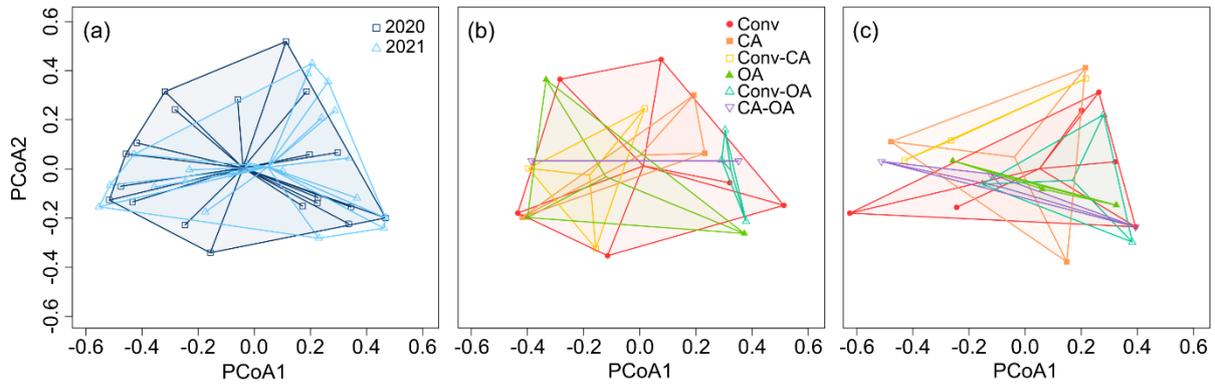


Figure S2.2. PCA on community-weighted mean of earthworm traits in (a) 2020 and (b) 2021. Ellipses represent different cropping systems. Conv, CA and OA: conventional, conservation and organic agriculture; Conv-CA, Conv-OA and CA-OA: initial-recent system transitions.

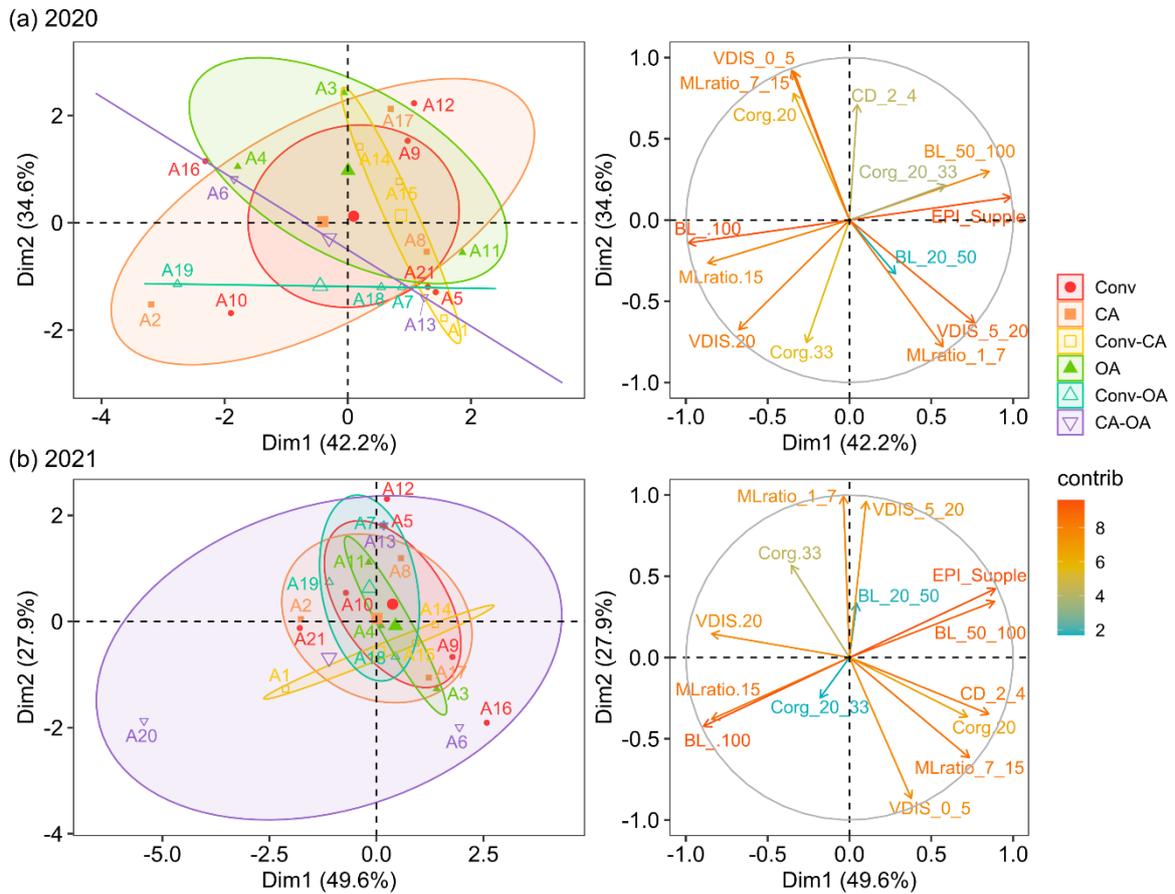


Table S2.1 Attribution of earthworm ecological categories and percentage of belonging to the three main categories after Bottinelli et al. (2020).

Species	Ecological categories	% epigeic	% anecic	% endogeic
<i>A. chlorotica</i>	Intermediate	31	31	38
<i>A. caliginosa</i>	Endogeic	16	4	80
<i>A. giardi</i>	Epi-anecic	30	70	0
<i>A. icterica</i>	Endogeic	0	8	92
<i>A. longa</i>	Epi-anecic	32	68	0
<i>A. rosea</i>	Endogeic	15	0	85
<i>L. castaneus</i>	Epigeic	90	10	0
<i>L. friendi</i>	Epi-anecic	34	66	0
<i>L. terrestris</i>	Epi-anecic	30	70	0

Table S2.2 Functional traits of earthworm species. Values are percentage of attribution to trait attributes.

Species	Body length (mm)			Body mass/length ratio (g.mm ⁻¹)			Cocoon diameter (mm)	
	20-50	50-100	>100	1-7	7-15	>15	2-4	4-6
<i>A. chlorotica</i>	0	99	1	0	100	0	100	0
<i>A. caliginosa</i>	0	100	0	67	33	0	60	40
<i>A. giardi</i>	0	0	100	0	100	0	67	33
<i>A. icterica</i>	0	100	0	0	100	0	73	27
<i>A. longa</i>	0	0	100	0	60	40	67	33
<i>A. rosea</i>	50	50	0	100	0	0	100	0
<i>L. terrestris</i>	0	0	100	0	0	100	33	67

Species	Epithelium type		Corg preferences (mg.kg ⁻¹)			Vertical distribution (cm)		
	supple	rigid	<20	20-33	>33	0-5	5-20	>20
<i>A. chlorotica</i>	99	1	18	38	45	60	40	0
<i>A. caliginosa</i>	100	0	16	38	47	20	70	10
<i>A. giardi</i>	0	100	22	34	43	33	33	33
<i>A. icterica</i>	100	0	13	51	35	10	80	10
<i>A. longa</i>	0	100	14	37	49	33	33	33
<i>A. rosea</i>	100	0	13	38	49	10	70	20
<i>L. terrestris</i>	0	100	13	43	45	40	40	20

Corg: organic carbon preferences.

Table S2.3 Earthworm species (a) density (ind.m⁻²) and (b) biomass (g.m⁻²) in cropping systems in 2020 and 2021.

	2020						2021					
	Conv	CA	Conv-CA	OA	Conv-OA	CA-OA	Conv	CA	Conv-CA	OA	Conv-OA	CA-OA
(a) Density (ind.m ⁻²)												
<i>Allolobophora chlorotica</i>	18 ± 24	32 ± 51	43 ± 46	30 ± 39	2 ± 3	14 ± 25	11 ± 17	12 ± 13	25 ± 22	27 ± 33	5 ± 9	27 ± 46
<i>Aporrectodea caliginosa</i>	24 ± 31	11 ± 9	7 ± 12	11 ± 14	80 ± 88	46 ± 80	16 ± 12	21 ± 28	11 ± 5	27 ± 11	25 ± 16	11 ± 18
<i>Aporrectodea giardi</i>	1 ± 2	2 ± 3	2 ± 3	4 ± 6	9 ± 11	2 ± 3	0 ± 0	0 ± 0	4 ± 6	0 ± 0	0 ± 0	2 ± 3
<i>Aporrectodea icterica</i>	4 ± 5	5 ± 9	11 ± 11	4 ± 6	2 ± 3	2 ± 3	1 ± 2	5 ± 9	16 ± 14	2 ± 3	0 ± 0	0 ± 0
<i>Aporrectodea longa</i>	4 ± 4	16 ± 23	2 ± 3	4 ± 6	16 ± 23	11 ± 14	4 ± 6	7 ± 8	9 ± 8	9 ± 3	5 ± 9	2 ± 3
<i>Aporrectodea rosea</i>	6 ± 10	7 ± 3	25 ± 20	0 ± 0	0 ± 0	9 ± 15	2 ± 4	4 ± 3	12 ± 11	0 ± 0	4 ± 6	0 ± 0
<i>Lumbricus castaneus</i>	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	2 ± 3	0 ± 0	0 ± 0
<i>Lumbricus friendi</i>	1 ± 2	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
<i>Lumbricus terrestris</i>	0 ± 0	0 ± 0	4 ± 6	0 ± 0	4 ± 3	2 ± 3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	2 ± 3	2 ± 3
(b) Biomass (g.m ⁻²)												
<i>Allolobophora chlorotica</i>	3 ± 4	6 ± 9	8 ± 9	6 ± 8	1 ± 1	5 ± 9	2 ± 3	3 ± 3	3 ± 3	5 ± 7	1 ± 2	7 ± 12
<i>Aporrectodea caliginosa</i>	10 ± 15	4 ± 4	2 ± 3	7 ± 11	29 ± 35	13 ± 23	5 ± 4	8 ± 11	3 ± 2	12 ± 12	7 ± 4	3 ± 5
<i>Aporrectodea giardi</i>	2 ± 5	2 ± 3	3 ± 5	4 ± 7	17 ± 22	2 ± 4	0 ± 0	0 ± 0	3 ± 5	0 ± 0	0 ± 0	6 ± 10
<i>Aporrectodea icterica</i>	3 ± 4	3 ± 6	7 ± 7	2 ± 3	1 ± 2	1 ± 2	1 ± 2	3 ± 5	8 ± 7	1 ± 1	0 ± 0	0 ± 0
<i>Aporrectodea longa</i>	8 ± 11	21 ± 30	2 ± 4	6 ± 10	22 ± 27	22 ± 31	4 ± 7	8 ± 9	14 ± 12	17 ± 6	7 ± 12	6 ± 10
<i>Aporrectodea rosea</i>	2 ± 3	1 ± 1	3 ± 2	0 ± 0	0 ± 0	1 ± 2	0 ± 1	1 ± 1	2 ± 2	0 ± 0	1 ± 1	0 ± 0
<i>Lumbricus castaneus</i>	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 1	0 ± 0	0 ± 0
<i>Lumbricus friendi</i>	3 ± 7	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
<i>Lumbricus terrestris</i>	0 ± 0	0 ± 0	5 ± 8	0 ± 0	12 ± 11	7 ± 12	0 ± 0	0 ± 0	0 ± 0	0 ± 0	3 ± 5	3 ± 5

Conv: conventional agriculture, CA: conservation agriculture, OA: organic agriculture, Conv-CA, Conv-OA and CA-OA: initial-recent system transitions.

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Figure S3.1 Pipeline used for processing the 16S and ITS2 data.

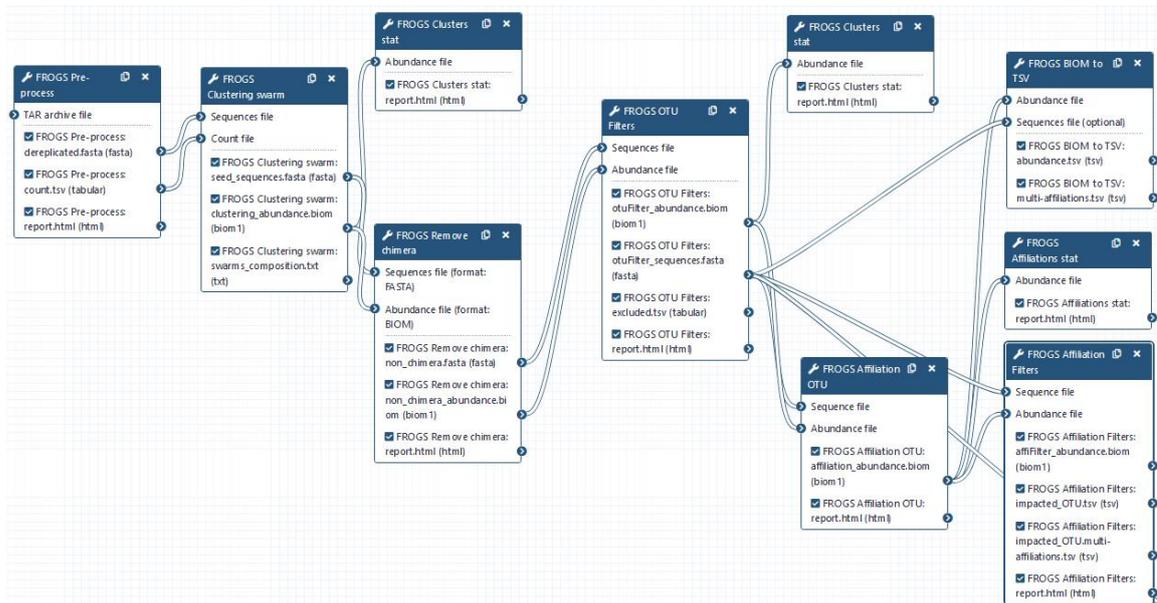


Figure S3.2 Hierarchically clustered heatmaps of the relative abundance of the main bacterial classes with plots ordered according to cropping systems. Only classes representing more than 1% of total abundance in at least one sample are presented. Columns correspond to plots named according to the cropping system they represent. Conv: conventional agriculture; CA: conservation agriculture; OA: organic agriculture; Conv-CA, Conv-OA and CA-OA: initial-recent system transitions.

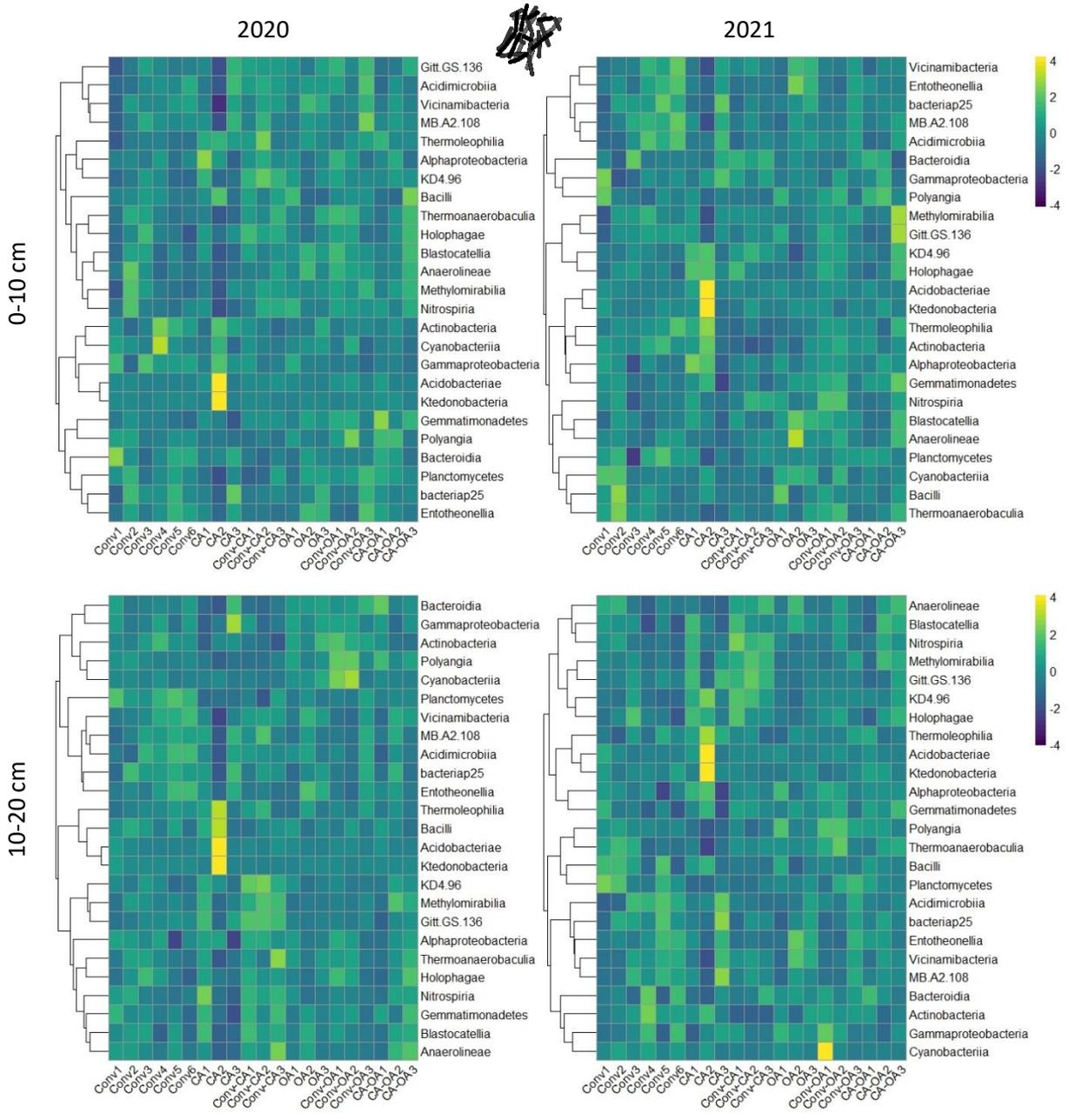


Figure S3.3 Hierarchically clustered heatmaps of the relative abundance of the main bacterial classes with plots ordered according to tillage intensity. Only classes representing more than 1% of total abundance in at least one sample are presented. Columns correspond to plots named according to the cropping system they represent. Conv, CA and OA: conventional, conservation and organic agriculture; Conv-CA, Conv-OA and CA-OA: initial-recent system transitions.

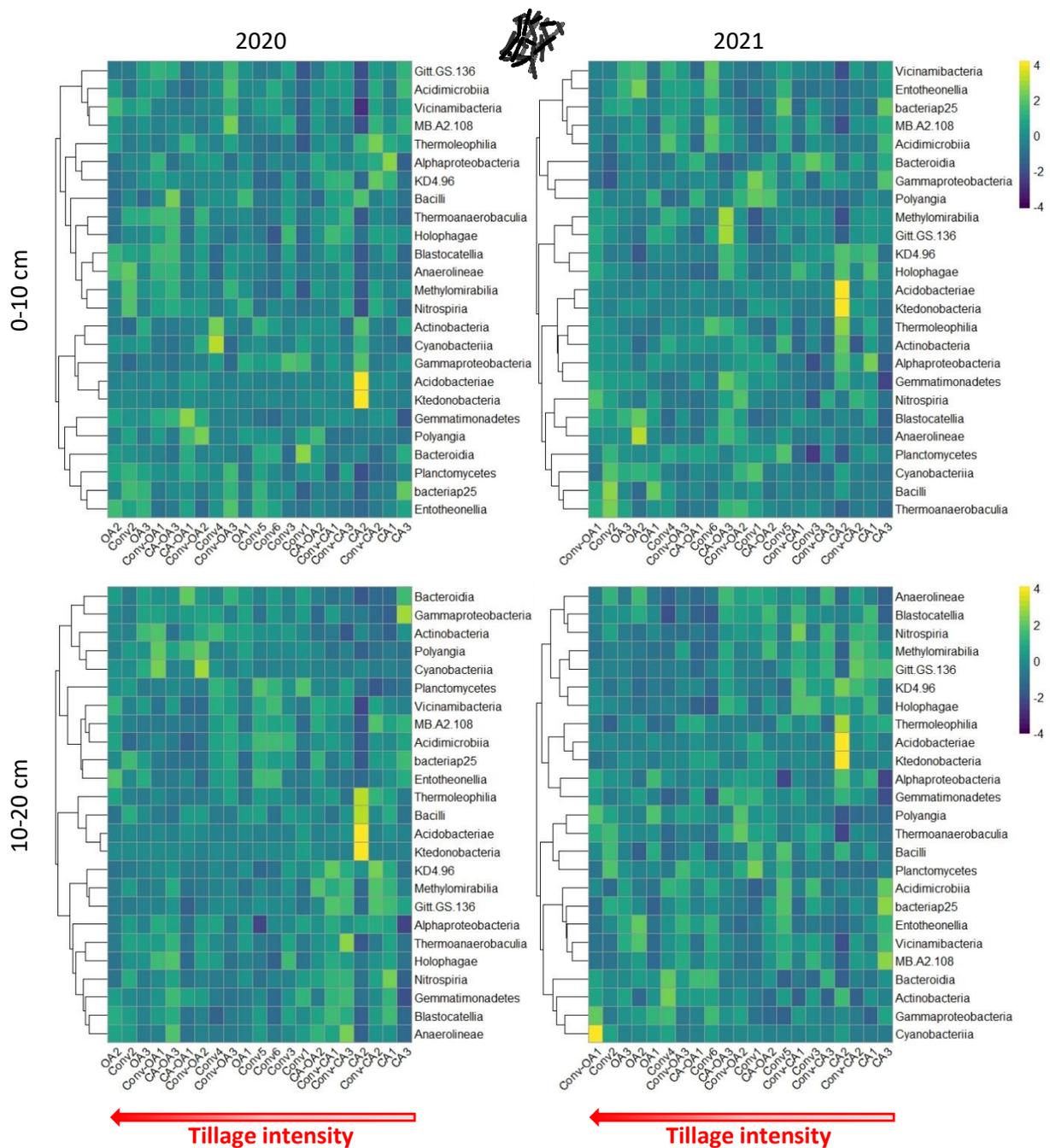


Figure S3.4 Hierarchically clustered heatmaps of the relative abundance of the main fungal classes with plots ordered according to cropping systems. Only class representing more than 1% of total abundance in at least one sample are presented. Columns correspond to plots named according to the cropping system they represent. Conv, CA and OA: conventional, conservation and organic agriculture; Conv-CA, Conv-OA and CA-OA: initial-recent system transitions.

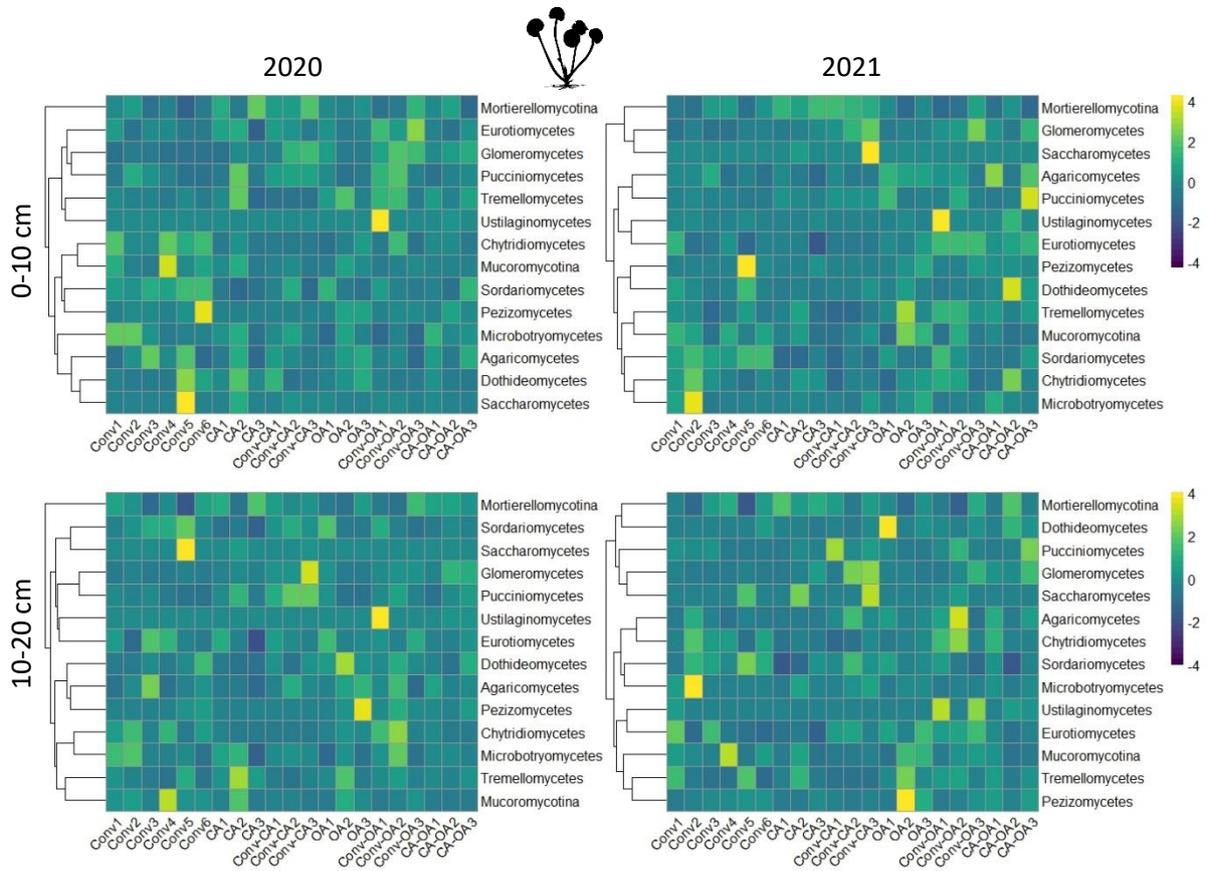


Figure S3.5 Hierarchically clustered heatmaps of the relative abundance of the main fungal classes with plots ordered according to tillage intensity. Only class representing more than 1% of total abundance in at least one sample are presented. Columns correspond to plots named according to the cropping system they represent. Conv, CA and OA: conventional, conservation and organic agriculture; Conv-CA, Conv-OA and CA-OA: initial-recent system transitions.

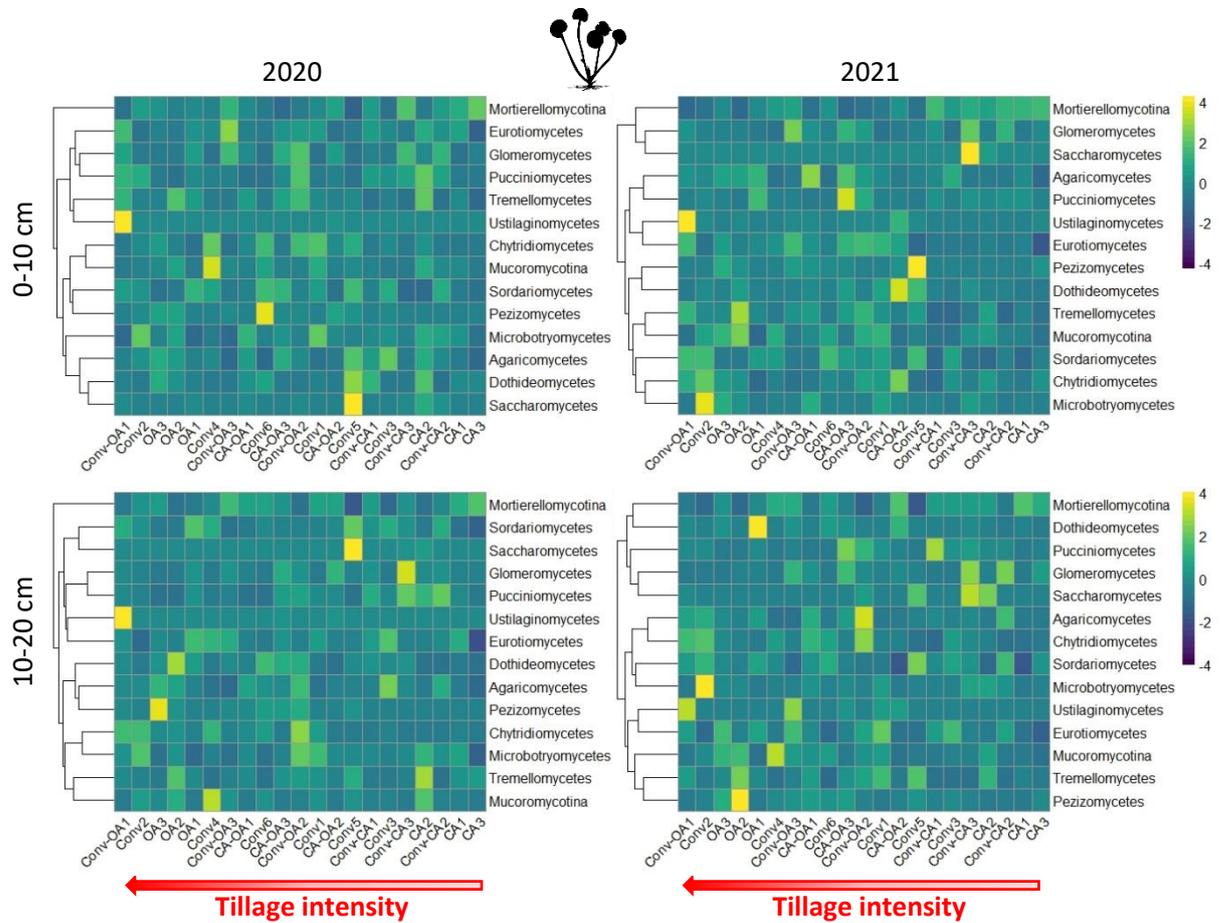


Table S3.1 List of all bacterial traits attributes influenced by cropping systems at (a) 0-10 cm and (b) 10-20 cm depth in 2020 or in 2021. Bold values indicate trait attributes showing similar differences between cropping systems at the same depth in both years. Traits were collected from the BactoTraits database. For more information see Cébron et al. (2021).

Depth	Trait attributes	
	2020	2021
(a) 0-10 cm	<p>ΔpH_5-9, ΔT_1-5, Trange_>40, Trange_<10, Topt_22-27, Motility_non-motile, Pigm_green, Pigm_white, Pigm_brown, Pigm_yellow, GC%_42-67, Shape_filamentous, Shape_rod, TT_methylotroph, TT_phototroph, TT_chemotroph, TT_organotroph</p>	<p>ΔpH_4-5, ΔpH_<1, pHrange_10-14, pHrange_8-10, pHrange_6-7, pHrange_4-6, ΔNaCl_1-3, ΔT_10-20, Trange_34-40, Trange_30-34, Trange_27-30, Gram_+, Pigm_white, GC%_>66, Width_0.5-0.65, TT_methylotroph, TT_organotroph</p>
(a) 10-20 cm	<p>ΔpH_1-2, NaClrange_1-3, ΔT_1-5, Trange_>40, Trange_30-34, Trange_22-27, Topt_>40, Topt_27-30, Topt_<10, Oxygen_anaerobic, Pigm_green, GC%_57-66, Shape_ovoid, Shape_filamentous, Shape_rod, TT_methylotroph, TT_phototroph, TT_chemotroph, TT_organotroph</p>	<p>pHrange_6-7, pHopt_8-14, , ΔNaCl_3-8, , ΔNaCl_1-3, NaClrange_3-8, NaClopt_3_8, NaClopt_<1, , ΔT_1-5, Trange_34-40, Trange_30-34, Topt_27-30, Pigm_green, Pigm_orange, GC%_>66, GC%_57-66, Length_>3, Width_0.5-0.65, Shape_filamentous, TT_phototroph, TT_organotroph</p>

Gram: gram staining; Width, Length, Shape: dimensions and shape of the cell; Pigm: colour of colonies; Motility: ability for motility; pHopt, pHrange, Δ pH: optimal, delta and range of pH for growth; NaClopt, NaClrange, Δ NaCl: optimal, delta and range of NaCl concentration for growth; Topt, Trange, Δ T: optimal, delta, range of temperature for growth; Oxygen: oxygen demand; TT: trophic type; GC%: GC content in the genome.

Appendix 4 - Supplementary material Chapter 4

Appendix 4.1 Initial conceptual model for path analysis used for analyses in R.

Initial model:

```
model<-'
SOM~Itill+Itreat+nbOrg
Q16S~Itill+Itreat+nbOrg+SOM+Earthworms
Q18S~Itill+Itreat+nbOrg+SOM+Earthworms
Macro.detritivores~Itill+Itreat+nbOrg+SOM+Q18S
Meso.detritivores~Itill+Itreat+nbOrg+SOM+Q18S+Earthworms
Earthworms~Itill+Itreat+nbOrg+SOM
Omnivorous~Itill+Itreat+nbOrg+SOM+Q18S+Q16S+Macro.detritivores+Meso.detritivores
+Meso.predators
Macro.predators~Itill+Itreat+nbOrg+Macro.detritivores+Meso.predators+Meso.detritivores
+Omnivorous+Earthworms
Meso.predators~Itill+Itreat+nbOrg+Meso.detritivores+Q18S'
```

Final model:

```
model<-'
SOM~Itill+Itreat+nbOrg
Q16S~nbOrg+SOM+Earthworms
Q18S~Itill+nbOrg+SOM
Macro.detritivores~Itreat+nbOrg+Q18S
Meso.detritivores~Itill+SOM+Q18S+Earthworms
Earthworms~Itill+nbOrg
Omnivorous~Itill+Macro.detritivores+Meso.detritivores+Meso.predators
Macro.predators~Itreat+Meso.detritivores+Omnivorous+Earthworms
Meso.predators~Itill+nbOrg+Meso.detritivores'
```

Figure S4.1 Effect size (Hedges' *g* estimate and 95% confidence interval) of stable or recent alternative cropping systems on the microbial biomass and the abundance of fungi and bacteria at 0-10 cm and 10-20 cm depth. Symbols represent the effect size obtained for the comparison between two systems (green circles = stable organic versus conventional, blue circles = recent organic versus conventional, orange triangles = stable conservation versus conventional, yellow triangles = recent conservation versus conventional, purple squares = recent organic from conservation versus stable conservation). Effect sizes are significantly positive or negative (*) when the confidence interval does not include zero. Conv, CA and OA: conventional, conservation and organic agriculture, Conv-CA, Conv-OA and CA-OA: initial-recent system transitions.

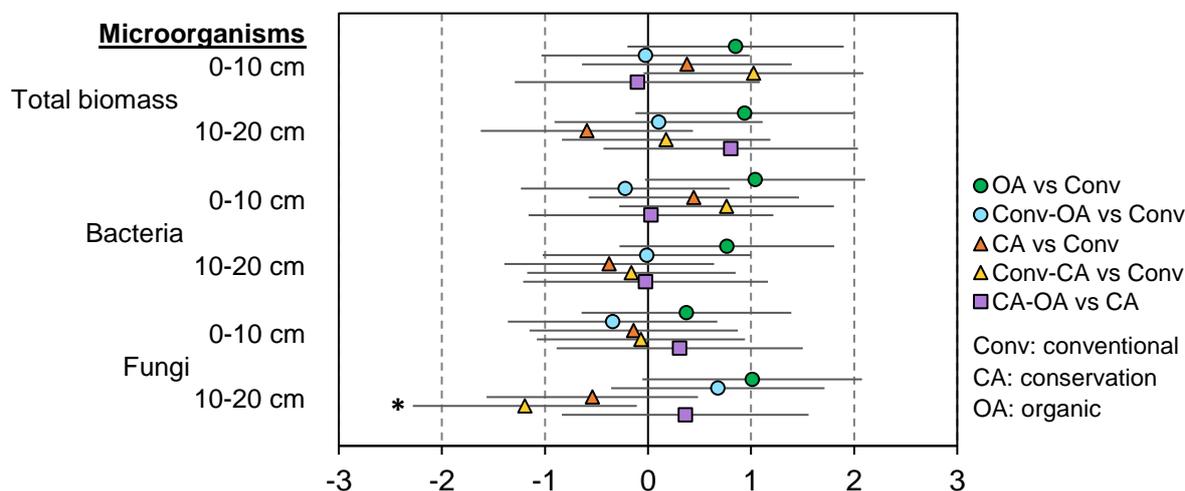


Table S4.1 Molecular microbial biomass ($\mu\text{g DNA.g soil}^{-1}$), bacterial abundance (10^9 16S copies.g soil $^{-1}$) and fungal abundance (10^8 18S copies.g soil $^{-1}$) at 0-10 and 10-20 cm depth in different cropping systems in 2020 and 2021. Values are means \pm standard deviations. Different lower-case letters indicate significant differences between cropping systems within a year according to the results of GLM with a quasi-Poisson error distribution ($P < 0.05$).

Variable	Depth	2020						2021					
		Conv	CA	Conv-CA	OA	Conv-OA	CA-OA	Conv	CA	Conv-CA	OA	Conv-OA	CA-OA
Microbial biomass	0-10	24 \pm 9	25 \pm 6	26 \pm 4	31 \pm 11	22 \pm 4	24 \pm 8	24 \pm 3	28 \pm 6	36 \pm 4	34 \pm 20	26 \pm 6	27 \pm 10
	10-20	24 \pm 8	16 \pm 3	24 \pm 5	31 \pm 11	26 \pm 9	23 \pm 1	23 \pm 6	23 \pm 4	26 \pm 4	34 \pm 17	23 \pm 8	24 \pm 4
Bacteria	0-10	12 \pm 4	11 \pm 3	11 \pm 1	14 \pm 3	10 \pm 2	12 \pm 3	11 \pm 1	15 \pm 2	16 \pm 1	16 \pm 6	11 \pm 2	14 \pm 4
	10-20	11 \pm 3	9 \pm 2	11 \pm 3	14 \pm 3	12 \pm 6	10 \pm 1	12 \pm 4	11 \pm 2	11 \pm 2	16 \pm 9	11 \pm 3	10 \pm 2
Fungi	0-10	6 \pm 4	5 \pm 1	3 \pm 1	5 \pm 2	4 \pm 2	5 \pm 1	4 \pm 2	5 \pm 1	7 \pm 2	8 \pm 3	4 \pm 1	6 \pm 3
	10-20	3 \pm 1	3 \pm 2	2 \pm 0	6 \pm 2	6 \pm 2	4 \pm 2	4 \pm 2	3 \pm 1	2 \pm 1	5 \pm 1	4 \pm 2	3 \pm 1

Conv, CA and OA: conventional, conservation and organic agriculture, Conv-CA, Conv-OA and CA-OA: transition from initial to recent system.

Table S4.2 Density (10^2 ind.m $^{-2}$) and trophic levels of soil mesofauna in cropping systems in 2020 and 2021. Values are means \pm standard deviations. Different lower-case letters indicate significant differences between cropping systems within a year according to the results of GLM with a quasi-Poisson error distribution ($P < 0.05$).

Taxa	Trophic group	2020						2021					
		Conv	CA	Conv-CA	OA	Conv-OA	CA-OA	Conv	CA	Conv-CA	OA	Conv-OA	CA-OA
Acari		310 \pm 313	232 \pm 97	95 \pm 88	42 \pm 25	29 \pm 7	90 \pm 39	138 \pm 119	231 \pm 34	235 \pm 85	232 \pm 248	113 \pm 65	255 \pm 145
		a	ab	ab	b	b	ab	a	a	a	a	a	a
Actinidida	Predator	1 \pm 2	6 \pm 7	13 \pm 23	1 \pm 0	0 \pm 1	7 \pm 3	4 \pm 5	0 \pm 1	6 \pm 11	0 \pm 1	0 \pm 1	9 \pm 8
		a	a	a	a	a	a	a	a	a	a	a	a
Gamasida	Predator	28 \pm 17	34 \pm 24	15 \pm 12	10 \pm 7	5 \pm 2	9 \pm 8	22 \pm 13	34 \pm 10	22 \pm 11	44 \pm 47	18 \pm 12	32 \pm 11
		a	ab	ab	ab	b	ab	a	a	a	a	a	a
Oribatida	Detritivore	280 \pm 305	192 \pm 91	67 \pm 56	30 \pm 28	24 \pm 8	73 \pm 48	113 \pm 109	197 \pm 43	207 \pm 95	188 \pm 201	94 \pm 61	214 \pm 126
		a	ab	ab	b	b	ab	a	a	a	a	a	a
Collembola	Detritivore	54 \pm 38	68 \pm 39	74 \pm 60	20 \pm 8	7 \pm 5	26 \pm 18	77 \pm 83	327 \pm 179	181 \pm 139	135 \pm 126	57 \pm 43	28 \pm 3
		a	ab	a	ab	b	ab	ab	a	ab	ab	ab	b

Conv, CA and OA: conventional, conservation and organic agriculture, Conv-CA, Conv-OA and CA-OA: transition from initial to recent system.

Table S4.3 Density (ind.m⁻²) and trophic levels of soil macrofauna in cropping systems in 2020 and 2021. Values are means \pm standard deviations. Different lower-case letters indicate significant differences between cropping systems within a year according to the results of GLM with a quasi-Poisson error distribution ($P < 0.05$).

Taxa	Trophic group	2020						2021					
		Conv	CA	Conv-CA	OA	Conv-OA	CA-OA	Conv	CA	Conv-CA	OA	Conv-OA	CA-OA
Araneae	Predator	9 \pm 12	23 \pm 13	11 \pm 9	7 \pm 3	4 \pm 6	2 \pm 3	36 \pm 22	36 \pm 3	16 \pm 11	23 \pm 11	18 \pm 22	16 \pm 14
Chilopoda		6 \pm 5	21 \pm 14	9 \pm 6	7 \pm 12	2 \pm 3	0 \pm 0	15 \pm 29	7 \pm 8	20 \pm 29	5 \pm 9	7 \pm 12	5 \pm 5
		ab	a	ab	ab	ab	b	a	a	a	a	a	a
<i>Geophilomorpha</i>	Predator	4 \pm 6	16 \pm 16	7 \pm 8	5 \pm 9	2 \pm 3	0 \pm 0	3 \pm 4	5 \pm 9	7 \pm 12	5 \pm 9	7 \pm 12	5 \pm 5
<i>Lithobiomorpha</i>	Predator	2 \pm 3	5 \pm 5	2 \pm 3	2 \pm 3	0 \pm 0	0 \pm 0	12 \pm 25	2 \pm 3	12 \pm 17	0 \pm 0	0 \pm 0	0 \pm 0
Coleoptera		25 \pm 20	80 \pm 30	64 \pm 46	48 \pm 38	27 \pm 11	34 \pm 20	43 \pm 45	82 \pm 40	85 \pm 44	50 \pm 13	59 \pm 56	69 \pm 46
<i>Carabidae</i>	Predator	9 \pm 12	14 \pm 16	11 \pm 11	18 \pm 16	7 \pm 8	7 \pm 8	11 \pm 6	14 \pm 3	34 \pm 13	12 \pm 6	18 \pm 8	21 \pm 11
		a	a	a	a	a	a	b	ab	a	b	ab	ab
<i>Curculionidae</i>	Plant feeder	3 \pm 3	5 \pm 5	4 \pm 3	2 \pm 3	0 \pm 0	0 \pm 0	2 \pm 4	0 \pm 0	0 \pm 0	0 \pm 0	2 \pm 3	4 \pm 3
<i>Staphylinidae</i>	Predator	6 \pm 9	4 \pm 3	5 \pm 5	7 \pm 8	11 \pm 0	2 \pm 3	19 \pm 38	18 \pm 11	20 \pm 8	14 \pm 13	5 \pm 5	16 \pm 19
<i>Other</i>	Various [§]	2 \pm 3	5 \pm 5	0 \pm 0	2 \pm 3	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0
<i>Larvae</i>	Omnivore	5 \pm 9	52 \pm 16	44 \pm 39	20 \pm 20	9 \pm 3	25 \pm 17	12 \pm 11	50 \pm 36	32 \pm 30	23 \pm 22	34 \pm 54	28 \pm 40
		b	a	ab	ab	ab	ab	a	a	a	a	a	a
Dermoptera	Omnivore	1 \pm 2	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	4 \pm 6	0 \pm 0	2 \pm 3	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0
Diplopoda		30 \pm 27	4 \pm 6	4 \pm 6	69 \pm 116	4 \pm 6	14 \pm 20	14 \pm 23	5 \pm 9	9 \pm 15	68 \pm 99	2 \pm 3	37 \pm 30
<i>Julida</i>	Detritivore	11 \pm 16	4 \pm 6	2 \pm 3	30 \pm 48	0 \pm 0	12 \pm 17	5 \pm 13	5 \pm 9	4 \pm 6	36 \pm 44	2 \pm 3	27 \pm 27
<i>Polydesmida</i>	Detritivore	20 \pm 28	0 \pm 0	2 \pm 3	39 \pm 68	4 \pm 6	2 \pm 3	9 \pm 22	0 \pm 0	5 \pm 9	32 \pm 55	0 \pm 0	11 \pm 5
Diplura	Predator	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	5 \pm 9	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0
Diptera (larv.)	Omnivore	4 \pm 4	12 \pm 22	2 \pm 3	0 \pm 0	0 \pm 0	0 \pm 0	1 \pm 2	2 \pm 3	5 \pm 5	0 \pm 0	0 \pm 0	2 \pm 3
Gastropoda	Plant feeder	0 \pm 0	7 \pm 12	2 \pm 3	2 \pm 3	0 \pm 0	0 \pm 0	4 \pm 6	20 \pm 13	12 \pm 11	9 \pm 8	4 \pm 6	2 \pm 3
Hemiptera	Plant feeder	0 \pm 0	0 \pm 0	2 \pm 3	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	4 \pm 3	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0
Isopoda	Detritivore	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	2 \pm 3	0 \pm 0	0 \pm 0	7 \pm 12	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0
Lepidoptera (larv.)	Plant feeder	0 \pm 0	5 \pm 9	2 \pm 3	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	2 \pm 3	2 \pm 3	0 \pm 0	0 \pm 0	0 \pm 0
Lumbricina	Various ^{§§}	252 \pm 58	284 \pm 161	343 \pm 140	148 \pm 98	340 \pm 155	281 \pm 301	206 \pm 121	196 \pm 80	402 \pm 113	212 \pm 147	404 \pm 220	233 \pm 258

[§]Dermestidae (Detritivore), Elateridae (Omnivore), Chrysomelidae (Plant feeder)

^{§§}Endogeic are mostly soil feeders whereas epigeic and anecic are litter feeders, but most species are opportunistic (Potapov et al., 2022)

Conv, CA and OA: conventional, conservation and organic agriculture, Conv-CA, Conv-OA and CA-OA: transition from initial to recent system, larv.: larvae.

Table S4.4 Estimate, standard error, z-value, P-value and standardized path estimates for the model use in path analysis. Bold values indicate significant effects ($P < 0.05$ *, < 0.01 **, < 0.001 ***).

Response	Predictor	Estimate	Standard error	z-value	P-value	Standardized estimate
SOM	Itill	-0.412	0.169	-2.443	0.015	-0.412
	Itreat	0.209	0.191	1.096	0.273	0.209
	nbOrg	0.710	0.186	3.826	<0.001	0.710
Q16S	Itill	-0.088	0.178	-0.498	0.619	-0.088
	nbOrg	0.281	0.182	1.546	0.122	0.281
	SOM	0.379	0.151	2.519	0.012	0.379
	Earthworms	-0.177	0.134	-1.317	0.188	-0.177
Q18S	Itill	0.141	0.186	0.755	0.450	0.141
	nbOrg	0.205	0.196	1.045	0.296	0.205
	SOM	0.246	0.163	1.510	0.131	0.246
Macrofauna detritivores	Itreat	0.319	0.180	1.768	0.077	0.318
	nbOrg	0.583	0.188	3.095	0.002	0.582
	Q18S	0.200	0.141	1.416	0.157	0.199
Mesofauna detritivores	Itill	-0.635	0.162	-3.910	<0.001	-0.637
	Itreat	0.128	0.169	0.759	0.448	0.128
	nbOrg	0.127	0.190	0.668	0.504	0.128
	SOM	-0.207	0.138	-1.499	0.134	-0.207
	Q18S	0.412	0.125	3.292	0.001	0.414
	Earthworms	-0.342	0.118	-2.902	0.004	-0.343
Earthworms	Itill	-0.329	0.180	-1.829	0.067	-0.329
	nbOrg	0.155	0.180	0.859	0.390	0.155
Macrofauna omnivores	Itill	-0.471	0.185	-2.546	0.011	-0.471
	nbOrg	0.119	0.186	0.641	0.521	0.119
	Macro. detritivores	0.165	0.155	1.061	0.289	0.165
	Meso. detritivores	-0.216	0.187	-1.158	0.247	-0.215
	Meso. predators	0.157	0.179	0.879	0.379	0.157
Macrofauna predators	Itreat	0.236	0.138	1.709	0.087	0.238
	Meso. detritivores	0.350	0.140	2.499	0.012	0.352
	Macro. omnivorous	0.178	0.131	1.351	0.177	0.179
	Earthworms	0.103	0.135	0.761	0.447	0.104
Mesofauna predators	Itill	-0.133	0.164	-0.814	0.416	-0.134
	Itreat	0.117	0.175	0.669	0.503	0.117
	nbOrg	0.201	0.170	1.180	0.238	0.201
	Meso. detritivores	0.568	0.134	4.250	<0.001	0.566

Appendix 5 - Supplementary material Chapter 5

Table S5.1 Results of ANOVA comparing the AIC of GLMM models with and without a fixed effect of climate, soil type, crop, agricultural system or litter on mass loss rate in (a) coarse, (b) medium and (c) fine mesh litterbags. Bold number indicate significant effect ($P < 0.1$).

	(a) Coarse mesh			(b) Medium mesh			(c) Fine mesh		
	χ^2	Df	<i>P</i> -value	χ^2	Df	<i>P</i> -value	χ^2	Df	<i>P</i> -value
Climate	5.70	3	0.13	7.38	3	0.06	1.84	2	0.40
Soil	21.74	8	0.005	35.83	7	< 0.0001	39.13	5	< 0.0001
Crop	8.94	3	0.03	12.63	5	0.03	15.59	5	0.008
System	0.95	3	0.81	2.09	4	0.72	0.35	3	0.95
Litter	24.81	11	0.01	22.80	6	0.0009	26.88	9	0.001

Table S5.2 Results of subgroup analysis on the effect size for categorical factors (climate, soil type, crop, agricultural system and litter) for (a) macrofauna, (b) mesofauna and (c) both macro- and mesofauna. Bold number indicate significant effect ($P < 0.05$).

	(a) Macrofauna effect			(b) Mesofauna effect			(c) Total fauna		
	QM	Df	<i>P</i> -value	QM	Df	<i>P</i> -value	QM	Df	<i>P</i> -value
Climate	44.19	3	< 0.0001	0.01	2	0.99	4.59	2	0.10
Soil	89.93	6	< 0.0001	2.94	4	0.57	12.00	2	0.002
Crop	42.95	4	< 0.0001	15.70	4	0.003	9.18	2	0.01
System	12.92	1	0.0003	5.78	2	0.06	0.97	1	0.32
Litter	6.10	3	0.11	4.69	1	0.03	6.84	2	0.03

Table S5.3 Results of subgroup analysis on the effect size for continuous factors (depth of bags, duration of the experiment and size of litter pieces) for (a) macrofauna, (b) mesofauna and (c) both macro- and mesofauna. Bold number indicate significant effect ($P < 0.05$).

	(a) Macrofauna effect		(b) Mesofauna effect		(c) Total fauna	
	QM	<i>P</i> -value	QM	<i>P</i> -value	QM	<i>P</i> -value
Depth	22.45	< 0.0001	0.001	0.98	0.21	0.64
Duration	2.14	0.14	9.75	0.002	6.88	0.01
Litter size	0.03	0.87	0.14	0.71	25.21	< 0.0001

Figure S5.1 (Enlargement of Figure 5.4) Effect size of macrofauna on litter decomposition. Represents the effect size of all the paired observations (n = 201) comparing coarse versus medium mesh litterbags (incl. /excl. macrofauna). "Mean effect size" represents the overall effect size of all paired observations. Black squares and bars represent the effect size of each paired observation and the associated 95% confidence interval. Numbers correspond to study numbers presented in Table 5.1.

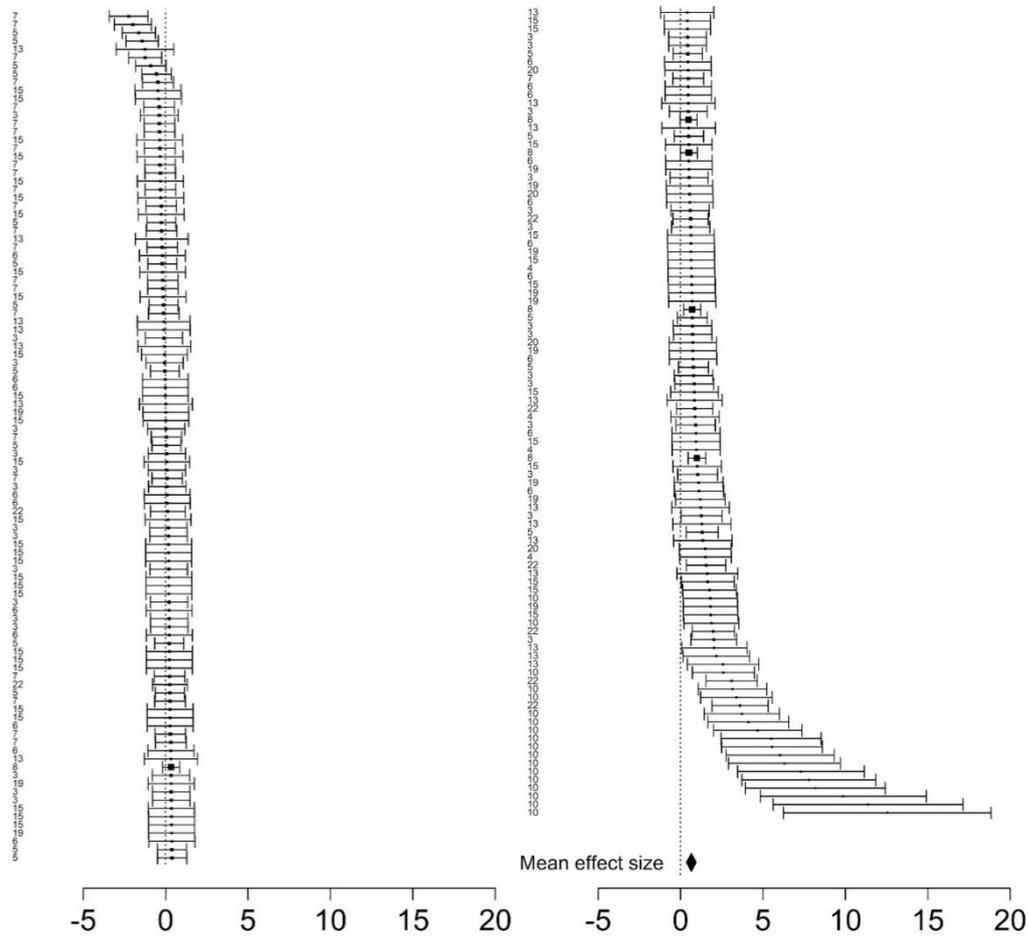


Figure S5.2 (Enlargement of Figure 5.5) Effect size of mesofauna on litter decomposition. Represents the effect size of all the paired observations (n = 126) comparing medium versus fine mesh litterbags (incl. /excl. mesofauna).

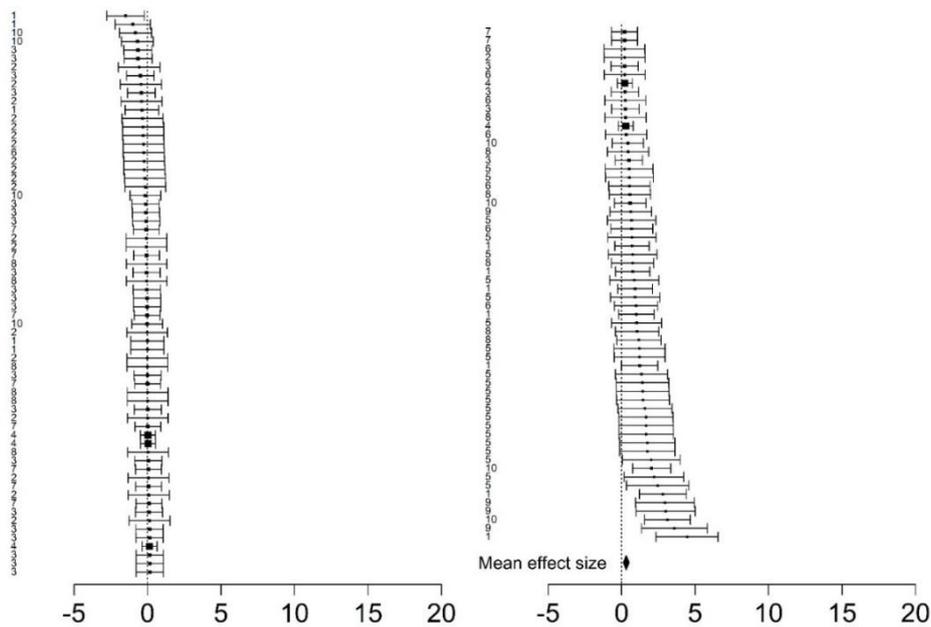


Figure S5.3 (Enlargement of Figure 5.6) Effect size of total fauna on litter decomposition. Represents the effect size of all the paired observations (n = 75) comparing coarse versus fine mesh litterbags (incl. /excl. macro- and mesofauna).

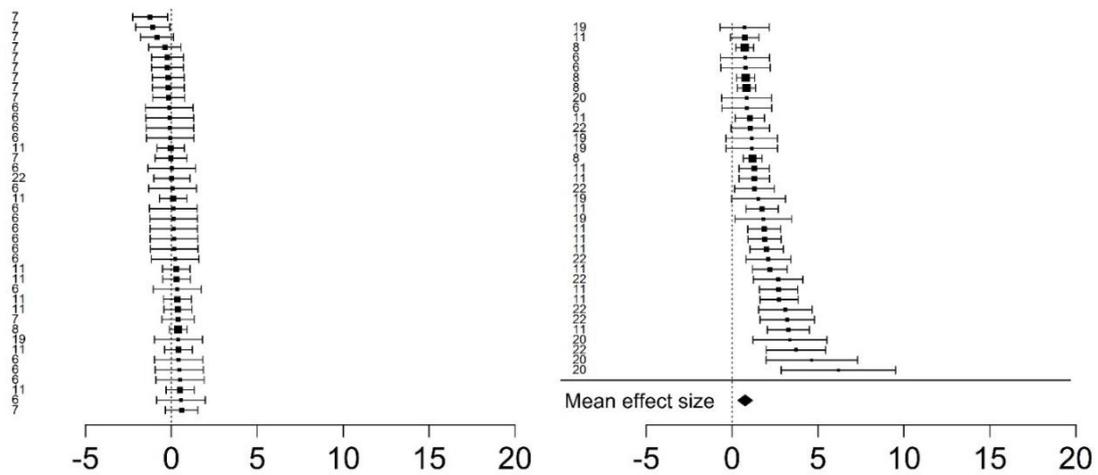
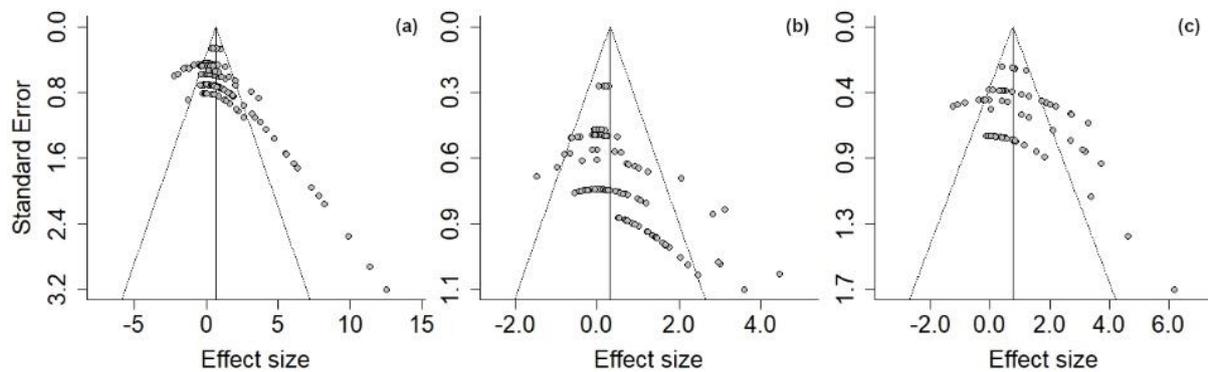


Figure S5.4 Funnel plot representing the publications bias of paired observations comparing (a) coarse versus medium mesh litterbags (incl. /excl. macrofauna), (b) medium versus fine mesh litterbags (incl. /excl. mesofauna), and (c) coarse versus fine mesh litterbags (incl. /excl. macro- and mesofauna). Each point represents one paired observation with its standard error and effect size.



Appendix 5.1 References list of the 17 studies used for the meta-analysis:

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- Zwahlen, C., Hilbeck, A., Nentwig, W., 2007. Field decomposition of transgenic Bt maize residue and the impact on non-target soil invertebrates. *Plant and Soil* 300, 245–257.

**Appendix 6 - Collembola are among the most pesticide-sensitive soil fauna groups:
A meta-analysis**

**Collembola are among the most pesticide-
sensitive soil fauna groups: A meta-analysis**

Sophie Joimel¹, Juliette Chassain¹, Maxime Artru¹, Juliette Faburé¹

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¹ Université Paris-Saclay, INRAE, AgroParisTech, UMR ECOSYS, 91120, Palaiseau, France

Critical Review

Collembola are Among the Most Pesticide-Sensitive Soil Fauna Groups: A Meta-Analysis

Sophie Joimel,* Juliette Chassain, Maxime Artru, and Juliette Faburé

UMR ECOSYS, INRAE, AgroParisTech, Université Paris-Saclay, Thiverval-Grignon, France

Abstract: Pesticides are a major concern because of their deleterious impacts on biodiversity and on the ecological functions provided by living organisms. Although earthworms are well studied, smaller-sized organisms, such as Collembola, also contribute to the agroecosystem functioning, and their sensitivity to pesticides makes them good bioindicators of soil quality. Using data from 21 publications, we performed a meta-analysis to compare the pesticide sensitivity of Collembola with other soil invertebrate groups and discuss the relevance of including tests on representatives of this microarthropods group in European regulation tests. We defined a paired observation as the median lethal concentration or the median effect concentration values for both Collembola species and another soil fauna group (Acari, enchytraeids, earthworms, isopods, and nematodes) under a unique combination of author, year, substance, and type of soil (61 and 57 paired observations for reproduction and lethal effects). In some studies, paired comparisons were available for several groups of soil fauna. We demonstrated that Collembola are among the most sensitive soil fauna groups to a variety of pesticides, notably for effects on reproduction, mostly compared with earthworms and enchytraeids. Because there are several modes of exposure and explaining factors, we suggest moving from a single-species study to a food-chain approach integrating different taxonomic groups. Differences between soil fauna groups in sensitivity or response to pesticides could have effects on soil communities and also on soil functions. *Environ Toxicol Chem* 2022;41:2333–2341. © 2022 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

Keywords: Regulation test; Ecotoxicological studies; Soil organisms; Collembola; Meta-analysis; Pesticides

INTRODUCTION

Conventional agriculture uses pesticides for plant protection all over the world, but this is a major concern due to their deleterious impacts on biodiversity and the ecological functions of living organisms. Regulatory ecotoxicity tests implemented in the pesticides regulation process are common and raise questions about which model organisms to use for these tests. Today, the role of earthworms in soils is well studied and described; earthworms are often used as a biological model in tests. Nevertheless, smaller-sized organisms also contribute to agro-ecosystem functioning and their sensitivity to pesticides makes them good bioindicators of soil

quality. This is particularly true of Collembola (Cortet et al., 1999). They are numerous in temperate agroecosystems and are an important trophic link in soil communities: on the one hand, they graze on microorganisms, on the other hand, they are consumed by a wide variety of predatory arthropods and by some insectivorous vertebrates. Their distribution and abundance in arable fields therefore influences the nutrient cycle and plant productivity, as well as the spatial disposition and abundance of their predators. Given their trophic position in agroecosystems, it is a significant challenge to maintain Collembola communities.

While Collembola are nontarget organisms of pesticide applications, they are often exposed to them (de Santo et al., 2018). Although less well known than earthworms, their diversity, their abundance in soils, and their ease of maintenance in laboratory conditions (short generation times) justifies their use in ecotoxicology. Although the first ecotoxicological studies on the effects of pesticides on Collembola date back to 1953, it was not until 2005 that the first comparisons were made between different taxa (Belden et al., 2005). Thus, many studies have already shown that Collembola are more sensitive to

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* Address correspondence to sophie.boulanger-joimel@agroparistech.fr

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certain pollutants than other soil organisms, such as earthworms, which are most often used for chemical assessments (Alves et al., 2014; Bandeira et al., 2020; de Lima e Silva et al., 2020). Frampton et al. (2006) demonstrated that the Collembola test species, *Folsomia candida*, is among the most sensitive species to pesticides and to a broad range of toxic modes of action (biocide, fungicide, herbicide, and insecticide) in lethality tests. This specific sensitivity to pesticides makes Collembola a good bioindicator of the pesticides ecotoxic effect.

The use of collembolans in toxicity testing is becoming more prevalent and relevant to soil toxicity testing. Experimental conditions in different studies on soil fauna can be similar if they follow the Organisation for Economic Cooperation and Development (OECD) guidelines for testing chemicals (OECD, 2016a) or International Organization for Standardization (ISO 11267:2014, 2014), which standardize the methodologies for ecotoxicological tests. Although *F. candida* is the most prevalent at this time, standardization efforts take into consideration an expansion of collembolan species (e.g., *Folsomia fimetaria*) available for use in ecotoxicity testing that captures other functional traits (e.g., sexual reproduction).

Moreover, earthworms and Collembola are not the only groups used as biological models for ecotoxicity tests. Efforts have also been focused on the diversification of soil fauna groups, including tests conducted on enchytraeids (OECD, 2016b) or Acari such as *Oppia nitens* (oribatid; Environment and Climate Change Canada, 2020) or *Hypoaspis aculeifer* (predatory; OECD, 2016c).

Nevertheless, the interpretation of the effects of pesticides on a soil community due to ecotoxicological tests remains quite complex. The lack of critical information for terrestrial invertebrate species hinders not only the establishment of environment soil quality criteria for contaminants in surface soils, but also a full risk assessment of the soil invertebrate community (Princz et al., 2018).

To promote the diversification of soil organism models for a pesticides ecotoxic effect, we carried out a meta-analysis of the sensitivity of several soil fauna groups to pesticides. The aim was to focus on the sensitivity of Collembola to pesticides compared with other soil invertebrate groups, and we discuss the relevance of this microarthropod in European regulation tests.

MATERIALS AND METHODS

Literature search

A systematic literature review was conducted on the basis of keywords in the ISI Web of Knowledge, using the "All Databases" option, with the following keywords: (springtail* OR folsomia* OR collembol*) AND ("pesticid* OR herbicid* OR insecticid* OR mollusc* OR nematocid*OR fungicid*) AND ecotox* in Topics (from 1955 to 2020).

A selection was made among a corpus of 260 references using titles and abstracts, and if necessary by examining the full text.

Pelosi et al. (2013) illustrate the value of a meta-analysis approach for comparing the sensitivity of different earthworm species to pesticides. Following this previous work on soil

fauna sensitivity to pesticides, we only considered publications which provided data on Collembola and any other taxonomic group of soil fauna in the same study to compare the sensitivity of the taxonomic group in similar conditions (e.g., type of substrate, possible addition of organic matter). We only included studies conducted on pesticides (in formulation or active substance) in laboratory tests conducted on natural or artificial soils and we excluded results from filter paper tests. By limiting the study to single-species tests, there were no restrictions on soil fauna groups or species to be included and our analysis was only limited by the availability of data.

It was also decided to focus on lethal effects and reproduction effects, avoiding behavior tests for which there were not enough studies which compared different soil fauna groups. Finally, we obtained a corpus of 21 publications but only 17 were used for data analysis (Supporting Information, Table S1). Indeed, several median lethal concentration (LC50) and/or median effect concentration (EC50) values were not available, either because of the absence of recorded values due to the lack of effect on the reproduction or survival of one or all the tested groups, or because of the absence of comparison under similar conditions.

Data extraction

The data from the 21 selected publications were entered into a database including several variables: author(s), year of the study, taxonomic group, species, active substance name, type of pesticide, pesticide addition method, soil type, and LC50 value or EC50 value with their standard deviation and replicates numbers.

When both standard deviation and confidence interval were missing for LC50 or EC50 values, the missing confidence interval was set equal to the largest one reported for LC50 or EC50 values in the studies selected for the same analysis. According to Pelosi et al. (2013), this approach allowed us to minimize the risk of underestimating the level of uncertainty associated with our calculations.

Data analysis

We defined a paired observation as the LC50 or the EC50 values for both a Collembola species and a species belonging to another soil fauna group under a unique combination of author, year, substance, and type of soil. Thus, when different modalities were reported in the same study, a paired observation was given for each modality to guarantee observation in the same conditions. In some studies, paired comparisons were available for several groups of soil fauna.

Finally, 17 publications were used to provide 61 and 57 paired observations for reproduction and lethal effect, respectively.

Effect sizes and 95% confidence intervals were calculated as the standardized mean difference between Collembola and other group values (i.e., for each paired observation) with a small-sample bias correction using Hedges' *g* calculation (Hedges & Olkin, 1985). The overall effect size was estimated

using a random-effect model. For an effect size to be considered as significantly positive or negative, its confidence interval should not cross the zero threshold. A negative effect size means that the studied soil fauna group is more sensitive to pesticides than Collembola, while a positive effect size indicates that Collembola have a higher sensitivity. The heterogeneity between studies was calculated using the I^2 statistic (Higgins & Thompson, 2002). Subgroup analyses were conducted to compare the sensitivity of Collembola with the sensitivity of others soil fauna groups (Acari, enchytraeids, earthworms, isopods, and nematodes), and for different pesticides (herbicides, insecticides, and fungicides) and soil types (natural and artificial) using relevant paired observations.

We performed all the statistical analyses using R Ver 3.5.0 (R Development Core Team, 2018) and the *metacont* package (Schwarzer, Carpenter, and Rucker, 2015).

RESULTS

Bibliometric analysis

In the 21 publications, the toxicity of pesticides on Collembola was compared mostly to earthworms (17 publications), followed by enchytraeids (10 publications), acari (five publications), and isopods (four publications). With only one publication each, comparisons with nematodes and gasteropods were also recorded. We also noticed that 50% of the studies make multiple comparisons (two, three, or four groups are sometimes compared with Collembola). On the other hand, there is little variability in the model species for each group (Table 1) whereas, except for imidacloprid (4 publications), the majority of pesticides have been tested only once. Most studies focused on the toxicity of insecticides and fungicides (Supporting Information, Table S1). The soil used for the experiments was mostly natural soil (13 publications), and more particularly LUFA soil. The artificial soil was Tropical Artificial Soil (TAS, 3) or OECD soils (6). Tropical artificial soil (pH 6.1, organic matter 5%) is adapted with low organic matter content from the OECD soil which is a loamy sand soil (pH 6.1, organic matter 10%). Tropical artificial soil also has finer sand than OECD soil (75% instead of 70%; de Santo et al., 2018).

TABLE 1: List of species in the 21 publications analyzed for comparison of the sensitivity to pesticides between Collembola and other soil fauna groups

Taxonomic groups	Species	Number of studies
Collembola	<i>Folsomia candida</i>	20
	<i>Lobella sokamensis</i>	1
Earthworms	<i>Eisenia fetida</i> (ss <i>andrei</i> or <i>fetida</i>)	17
Enchytraeids	<i>Enchytraeus crypticus</i>	9
	<i>Enchytraeus albidus</i>	1
Acari	<i>Hypoaspis aculeifer</i>	3
	<i>Oppia nitens</i>	2
Isopods	<i>Porcellio scaber</i>	3
	<i>Armadillidium</i> sp.	1
Nematodes	<i>Caenorhabditis elegans</i>	1
Gasteropods	<i>Helix aspersa</i>	1

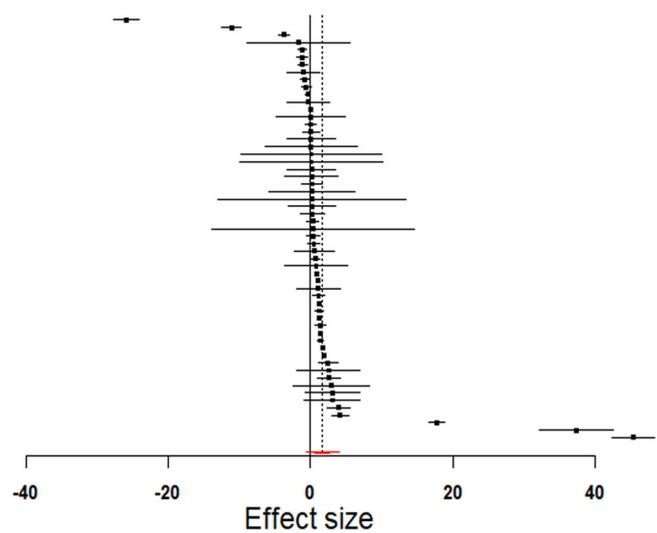


FIGURE 1: Comparison of the sensitivity to pesticides on the lethality of Collembola and other soil fauna groups. Shown is the effect size defined as the difference in median lethal concentration values (black point) and corresponding confidence interval (95%; black line) of all paired observations on lethality effects. Mean effect size (red point) and confidence interval (red line) were calculated from the 57 experimental paired observations on lethality.

Lethal effect

The 95% confidence intervals of the individual effect size frequently overlap the zero threshold (Figure 1), and the overall pooled effect size value (computed over all groups) was not significantly positive (1.7, confidence interval = -0.6 to 4.0 ; Table 2) indicating that Collembola species were not significantly more sensitive to pesticides than other species. A substantial heterogeneity was observed ($I^2 = 98.5\%$).

Due to the high variability, the mean effect sizes and their confidence intervals confirm only that enchytraeids are significantly less sensitive to pesticides than Collembola. No difference in sensitivity is due to soil type (natural or artificial soil). However, we noticed a higher sensitivity of Collembola to insecticides (3.0) than to fungicides (-2.7), with a significant effect size for insecticides only (Table 2). The number of paired

TABLE 2: Comparison of the sensitivity to pesticides on lethality of Collembola and other soil fauna groups

	k	SMD	95% CI
All groups	57	1.7	-0.6 to 4.0
Groups			
Acari	17	2.8	-1.4 to 7
Enchytraeids	7	1.0	0.9 – 1.1^a
Earthworms	27	-0.5	-3.0 to 2.0
Pesticides			
Fungicide	13	-2.7	-6.9 to 1.5
Insecticide	44	3.0	0.4 – 5.7^a
Soil type			
Natural	45	1.6	-0.3 to 3.4
Artificial	12	1.8	-7.0 to 11.0

^aSignificant difference.

Shown are the number of paired observations (k), the standardized mean effect size (SMD) defined as the difference in median lethal concentrations between Collembola and the mentioned soil fauna group, and the corresponding 95% confidence intervals (95% CI).

TABLE 3: Comparison of the sensitivity to pesticides on the reproduction of Collembola and other soil fauna groups

	<i>k</i>	SMD	95% CI
All groups	61	3.5	2.0–5.0 ^a
Groups			
Acari	19	1.3	1.0–1.6 ^a
Enchytraeids	14	4.1	1.9–6.2 ^a
Earthworms	24	4.9	1.3–8.4 ^a
Pesticides			
Fungicide	19	7.0	2.5–11.4 ^a
Insecticide	34	2.6	2.4–2.9 ^a
Soil type			
Natural	47	4.3	0.8–1.5 ^a
Artificial	14	1.2	2.4–6.2 ^a

^aSignificant difference.

Shown are the number of paired observations (*k*), the standardized mean effect size (SMD) defined as the difference in median effect concentrations between Collembola and the mentioned soil fauna group, and the corresponding 95% confidence intervals (95% CI).

observations was too small for other soil fauna groups (i.e., gasteropods and isopods) or other variables (e.g., active substances) to justify separate analyses.

Reproduction effect

Most of the individual effect sizes were higher than zero (56 paired observations out of 61), and the mean effect size for reproduction effect was 3.5 (confidence interval = 2–5; Table 3), indicating that Collembola species are generally more sensitive to pesticides than the other soil fauna groups. A high heterogeneity was also observed ($I^2 = 98\%$).

The mean effect sizes and their confidence intervals confirm that earthworms and enchytraeids, two common soil fauna groups in ecotoxicological tests, were significantly less sensitive to pesticides than Collembola (Table 3 and Figure 2).

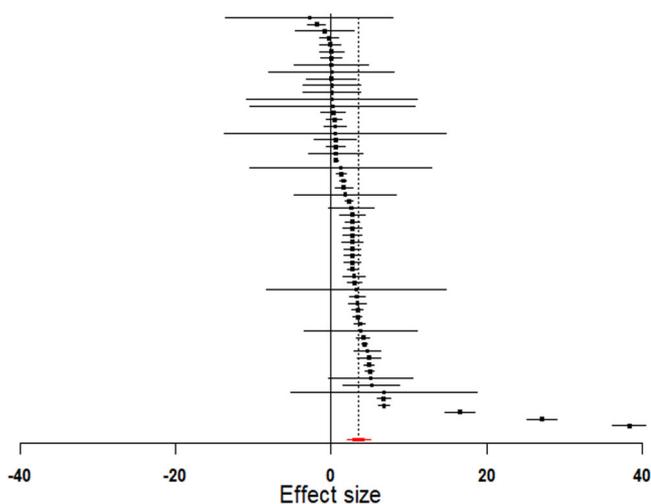


FIGURE 2: Comparison of the sensitivity to pesticides on the reproduction of Collembola and other soil fauna groups. Shown is the effect size defined as the difference in median effect concentration values (black point) and corresponding confidence interval (95%; black line) of all paired observations on reproduction effects. Mean effect size (red point) and confidence interval (red line) were calculated from the 61 experimental paired observations on reproduction.

However, the mean effect size obtained for Acari (soil fauna groups less commonly used in ecotoxicological studies) showed similar sensitivity to pesticides for Collembola. Concerning the effect of other variables, such as pesticide type or soil type, we noticed a higher sensitivity of Collembola in natural soils than in artificial soils and a higher sensitivity to fungicides than to insecticides (confidence interval = 2.5–11.4). The number of paired observations was too small for other soil fauna groups (i.e., gasteropods and nematodes) or other variables (e.g., active substances) to justify separate analyses.

DISCUSSION

The corpus of studies selected for this meta-analysis allowed us to compare the sensitivity of several soil fauna groups to pesticides. While the registration procedure for plant protection products requires data from industry, these are usually confidential. Moreover, because pesticide regulation favors a predictive approach, the data collected were generated in standardized toxicity tests performed under controlled conditions. For these reasons, our corpus consists of only 21 publications. It is nevertheless enough to demonstrate that Collembola are among the most sensitive soil fauna groups to a variety of pesticides, notably with effects on reproduction.

The LC50 data show a higher sensitivity of springtails to insecticides than to fungicides. This is consistent with the literature, which generally describes a high sensitivity of terrestrial invertebrates to insecticides (Pekar, 2012; Pekar & Benes, 2008). However, it should be noted that the ecotoxic effect of insecticides is studied more often than that of fungicides, and our corpus confirms this because 11 papers concern insecticides against only four for fungicides.

However, our EC50 meta-analysis shows a higher sensitivity of springtails to fungicides. This result is consistent with the study of Christensen and Mather (2004), which showed an increase in the surface activity of springtails exposed to fungicide-treated seeds. In contrast, the study led by Daam et al. (2011) tends to describe different results from this analysis. While springtails appear to be more sensitive than the other biological models used in our analysis, Daam et al. describe a higher sensitivity of *Eisenia fetida* to fungicides than springtails. This difference can be explained by several elements related to the study protocol: Daam et al. compared the sensitivities of the biological models based on no-observable effect concentration data and by calculating the Species Sensitivity Distribution. In addition, their dataset was composed of data from the US Environmental Protection Agency ECOTOX database, and included 21 data for insecticides compounds and seven for fungicides. Also, the Collembola data concerned nine different taxa whereas the data collected in our meta-analysis concern mainly the species *F. candida*. Finally, Daam et al. (2011) sought to characterize the difference in sensitivity of *E. fetida* compared with other models of terrestrial invertebrates, whereas our approach is focused on springtails. All these methodological differences greatly limit the comparison of the results obtained by Daam et al. (2011) with our own.

The observed differences in sensitivity of soil fauna to pesticides are difficult to explain (Neuhauser et al., 1986) but could have important consequences for soil functioning (Bart et al., 2017). These factors could be related to their morphology, physiology, or ecological parameters (behavioral characteristics) inducing a higher exposure depending on the characteristics of soil organisms.

A first obvious hypothesis would be to link sensitivity to the size of the organisms. If several authors have put forward that a larger size involves a larger surface responsible for a higher exposure, this hypothesis has often been refuted, especially in the case of the differences in sensitivity found between earthworm species (Ma & Bodt, 1993; Pelosi et al., 2014). Indeed, small organisms have surface/volume ratios higher than larger organisms, which involves a higher exposure due to their low biomass. In both cases, this relationship between size and difference in sensitivity must be questioned because (1) earthworms are larger and less sensitive than Collembola, and (2) within the same-size group (e.g., mesofauna) differences in sensitivity are observed between Collembola and enchytraeids. The size criterion is especially important if the exposure is mainly by contact.

For this reason, another hypothesis, still related to morphology, has been put forward that emphasizes the characteristics specific to each group that influence their mode of exposure. According to Alves et al. (2014), although earthworms may be exposed through more different pathways to contaminants in soil, in comparison to Collembola, the toxicity of the substances can be conditioned not only by the route of exposure, but mainly by its mode of entry. For example, the ventral tube of Collembola, the colophore, would be an additional route of absorption for toxic substances, in addition to ingestion via nutrition (Fountain & Hopkin, 2005). In contrast, soft-bodied organisms (earthworms, enchytraeids) are more likely to be exposed via passive skin penetration of pore water (Belfroid et al., 1994), in addition to ingestion. Depending on their chemical properties (e.g., more or less soluble in water or fat), pesticides may be preferentially found in the solid or aqueous phase of the soil. Because exposure pathways differ between taxonomic groups, these chemical properties may be the reason for preferential exposure of one taxon over another. That explains the differences in sensitivity of organisms to pesticides: in this case, this apparent difference in sensitivity would indirectly reflect the variability of the organisms' exposure routes to pesticides.

In addition, the interspecies variations of pesticide distribution, biotransformation, and elimination are likely involved in the variability of pesticide sensitivity. This difference in absorption route is particularly important because it conditions toxicokinetics and more specifically the metabolization phase of substances, which can be different according to this absorption route. Soil organisms have a well-established enzyme system that detoxifies organic pesticides compounds taken up by the organism but differ between taxonomic groups (Van Straalen, 1993). Also, isopods and Collembola are highly efficient in biotransforming organic chemicals whereas earthworms seem less efficient. While this can provide better

resistance to certain chemicals, it can also cause a possible consequence of this rapid biotransformation, because potentially toxic metabolites may be produced (van Gestel, 2012).

Concerning physiology, explanatory factors can be found in the capacity of organisms or species to adapt to a contaminated environment through physiological adaptations (Fitzgerald et al., 1996). Chronic exposure to pesticides for generations may allow adaptation by favoring individuals that face them (Givaudan et al., 2014). Physiological adaptation, which induces, for example, a better ability to detoxify chemicals, may lead to genetic adaptation if it implies hereditary mechanisms. Genetic adaptation could also pass on the selection of genotypes allowing a better tolerance to chemicals by avoiding exposure (e.g., digging deeper or maturing faster; Givaudan et al., 2014). Such adaptations in terrestrial invertebrates have been demonstrated for metals (e.g., Gudbrandsen et al., 2007; Posthuma & Van Straalen, 1993) and also for pesticides.

For example, Givaudan et al. (2014) have demonstrated the pesticide acclimation of an earthworm population from conventionally cropped fields. Compared with the responses measured of earthworms from organic farming, the exposure to the agricultural fungicide epoxiconazole induced an increase in burrowing behavior and a higher detoxification rate in earthworms from conventional farming. This means that the different explanatory factors are not independent and that other responses of organisms, such as cast production and burrowing, can be observed rather than lethality or reproduction responses and have consequences on the ecosystem functioning.

A functional trait approach, which has been extensively developed in ecology (Pey et al., 2014), particularly in Collembola (e.g., Joimel et al., 2018) and earthworms (Pelosi et al., 2014), could help to better highlight the explanatory factors. Moreover, these traits also take into account behavioral abilities, which were not included in our study. Tests could thus be carried out on other effects of pesticides to give a holistic view of the effects of pesticides on soil fauna, for example on the movement of organisms or on pesticide avoidance. This type of test remains rare (Ximenes et al., 2020) but see, for example, Niemeyer et al. (2018), who carried out several studies on behavior. It is also important to note that avoidance tests are already used in ecotoxicology.

Differences in sensitivity between taxonomic groups could also be explained by the different modes of action of molecules. Indeed, the neurotoxic molecules of neonicotinoids, for example, have an affinity for insects, to which Collembola are more related than earthworms (Akeju, 2014).

It has also been hypothesized that proteins within the chitinous exoskeleton of some arthropods may increase the potential for bioaccumulation (Prosser et al., 2016). Alternate sources of exposures may include a contamination of primary food sources for Collembola, fungi, which could absorb pesticides within the soil environment (Princz et al., 2018).

Nevertheless, a high degree of heterogeneity in sensitivity is observed between studies. The variation in the experimental conditions is often put forward to explain data variability between different studies, but this does not seem to be the case in the studies reviewed.

Indeed, the experimental conditions of the different studies are very similar because they follow the OECD guidelines for testing chemicals (OECD, 2016a), which standardize the methodologies for ecotoxicological tests. The high variability could therefore be explained mainly by the multitude of molecules tested and the fact that the substances are mostly tested only once on several taxonomic groups.

The variability observed could also be explained by other factors, such as the type of soil and differences in its composition and structure. Indeed, the amount of organic matter in the soil, and its type, would influence the bioavailability of pesticides (Ogungbemi & van Gestel, 2018). Similarly, the soil texture (proportion clay/sand/silt) seems to influence the bioavailability of molecules (Bandeira et al., 2020) and the soils organisms' exposure to the substance. For example, EC50 values in soils with high clay and silt content (>90%) were twice as low as those found in soils with <20% of these elements, with the same organic matter content (Bandeira et al., 2020).

Soil risk assessment, remediation, and contaminant management strategies should represent the soil functional aspects (Princz et al., 2018). To meet part of this challenge, a soil toxicity testing approach has been developed on a diversity of species: earthworms, Collembola, Acari, and more recently enchytraeids, nematodes, and isopods. However, it is obvious that the current battery is not complete and also not well balanced (van Gestel, 2012). Earthworms and Collembola are the most used species for tests, as models of nontarget macro- (Pelosi et al., 2014) and mesofauna (Amorim et al., 2012; de Santo et al., 2018). It is therefore not surprising that Collembola are mostly compared with earthworms in our study. Collembola inhabit various organic substrates and use a wide range of food sources. They are also involved in the food chain basis for other species, acting as nutrient cycling catalyzers as well as changing the soil structure through litter comminution, casting, and other mechanisms (de Santo et al., 2018; Domene et al., 2011; Potapov et al., 2016). As for earthworms, they are responsible for a large fraction of the biomass in the soil, they play an important role in the functioning of the soil ecosystem (de Santo et al., 2018), and they are considered as ecosystem engineers due to their action on the soil structure and nutrient cycling (Lavelle & Spain, 2003). Earthworms and Collembola are considered to be key groups in the soil ecosystem, but this is also the case for other groups such as Acari or isopods, and many justifications for their use are also given in review (Castro-Ferreira et al., 2012; Hägerbäumer et al., 2015; Lebrun & van Straalen, 1995; van Gestel et al., 2018). Moreover, most ecotoxicological studies mainly focus on earthworms to assess the ecotoxicity of the compounds (Alves et al., 2013; Pelosi et al., 2014), but they may not be ideal indicators of the risks posed to terrestrial fauna by insecticides and other similar substances (Alves et al., 2013; Jänsch et al., 2006). In our study, the standard earthworm test species, *E. fetida sensu lato*, seems to be less sensitive than Collembola when considering the reproduction data and in Frampton et al. (2006) it is the least sensitive species to insecticides based on acute mortality, whereas the standard Collembola test species, *F. candida* (Willem, 1902), is among the most sensitive species to a broad

range of toxic modes of action (biocide, fungicide, herbicide, and insecticide). However, although *F. candida* is faced with little criticism, it is different for *E. fetida*, whose use in ecotoxicological tests is often decried and would benefit from a replacement by *Apporectodea caliginosa*, a more sensitive and common species in agricultural land use (e.g., Pelosi et al., 2013).

To conserve soil biodiversity, some regulations still use lethality to assess substance toxicity in regulation tests (see, for example, Brazil and Argentina where mostly lethality tests are used on earthworms [Camargo Carniel et al., 2019] which is not the best parameter to provide accurate information [Cortez et al., 1999]). This parameter does not respond strongly to substance toxicity with few differences between taxonomic groups compared with the response of soil organisms in terms of reproduction. Within the ecotoxicological tests, sublethal endpoints (i.e., burrowing activity, acute toxicity, cast production, avoidance, biomarkers, survival, and reproduction) are more relevant to assess the toxicity of chemical products (de Lima e Silva et al., 2020). Specifically, reproduction is a more relevant endpoint when translating effects to the population level (van Gestel, 2012). Nevertheless, the development of subindividual biomarkers could be even more interesting for the early detection of the sensitivity of springtails to relatively low pesticide concentrations. For instance, Saha and Joy (2016) showed a strong biochemical impact of agriculturally recommended doses of insect growth regulator on tissue nutrient levels and digestive enzyme activities in *Cyphoderus javanus* within 7 days of exposure in microcosms. Although subindividual biomarkers are not widely used in regulatory environmental risk assessment, the precocity of their response makes them relevant tools for assessing the potential ecotoxicity of pesticides.

Some studies have demonstrated that in each taxonomic group there are differences in sensitivity, which could be explained by many factors (e.g., in earthworms Pelosi et al., 2013), including reproduction mode and strategy. Collembola have been used since the beginning of the 1960s as a model organism for assessing the toxicity of chemicals in soils (de Lima e Silva et al., 2021). Species models are reduced to the species *F. candida*, which is the most studied, whereas other species are recommended notably by OECD standardized tests (as *F. fimetaria*) or the Canadian Ministry for Environment and Climate Change (*Proisotoma minuta*). However, studies about the indicating value of this parthenogenetic species (*F. candida*) for species of Collembola which reproduce sexually are very recent (de Lima e Silva et al., 2021). Reproduction strategy (*r* or *k* strategy, for example) could also play a role in the differences in responses to pesticides in reproduction tests for different soil organisms. Organisms that focus on a reproductive strategy, especially when confronted with stress, may show less effect in terms of reproduction than a species focusing on growth. Also, the differences observed may be explained by differences in stress responses, rather than differences in sensitivity to pesticides. Moreover, Frampton & van den Brink (2007) show differences in sensitivity to insecticides between springtails species and explain that the in situ assessment approach based on total community abundance does not identify taxon-specific

effects. They therefore recommend targeted monitoring of representative and sensitive species, such as *Entomobrya multifasciata*.

Finally, since the introduction of the term ecotoxicology, the question of “putting more eco into ecotoxicology” has been raised (van Gestel, 2012). This notion has triggered the focus on more ecologically relevant test designs, integrated approaches including responses at different levels of biological organizations, and taking into account the normal operating range of parameters describing the structure and functioning of soil ecosystems (van Gestel, 2012). The aim is to validate the laboratory studies under more realistic conditions. To meet this challenge, more complex issues have been highlighted, including ecological vulnerability, trait-based analysis, and effects on functional endpoints (so-called ecosystem services; van Gestel, 2012). This is why it is crucial to carry out soil toxicity tests on a diversity of endpoints and species/groups. In addition, biotic interactions will definitely need more attention in the future and so will long-term effects involving several generations (Filser et al., 2014). Current environmental guidelines in Europe use single-species data in environmental protection tests. Today, it is of high importance to develop a method that can easily derive a community effect. Renaud et al. (2021) suggested incorporating community data into the assessment of the heavy metal effect on the environment. They used community similarity dose–response curves to measure community effects and demonstrate their potential for inclusion in risk assessment schemes as a measure of community response (Renaud et al., 2021). In the same way, the intra-community variability should be considered to assess the pesticide effects on Collembola. Thus, Fountain et al. (2007) showed that the addition of the insecticide chlorpyrifos to the soil decreased the diversity and species richness of springtails, but the total abundance of springtails increased. The springtail *Ceratophysella denticulata* was found to dominate the overall community. In addition, Frampton & van den Brink (2007) showed that the sensitivity of Collembola prey species could differ from that of its predator species due to indirect effects of pesticides. This is the reason why the pesticide regulation process should take into account the community responses to chemicals. The goal would be to improve knowledge of trophic interactions to better understand the direct and indirect effects on organisms and consequences on the scale of communities and ecological functions. This is required to provide adequate environmental protection.

CONCLUSION

Soil risk assessment, remediation, and contaminant management strategies should represent soil functional aspects. To meet this challenge, our results promote the diversification of soil organism models for pesticides ecotoxic effects. Indeed, in using the case of Collembola, we demonstrated that different soil organism models have different responses to pesticides. Collembola are among the most sensitive soil fauna groups to a variety of pesticides, notably with effects on reproduction.

Because Collembola are closer to insects than earthworms, they could be a good indicator of insecticide effects.

Because there are several modes of exposure, and cascading effects that are possible, there is a challenge to move from a single-species study to food chains integrating different taxonomic groups. Their differences in sensitivity or response could have effects on soil communities but also on soil functions. New soil organism models need to be tested to complement the test batteries that still rely too heavily on earthworms.

Supporting Information—The Supporting Information is available on the Wiley Online Library at <https://doi.org/10.1002/etc.5428>.

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— Résumé étendu de la thèse en français —

Introduction

Le sol contient une biodiversité insoupçonnée qui représente 25% des espèces décrites dans les écosystèmes terrestres (Decaëns et al., 2006). Les organismes du sol interagissent, participent à de nombreuses fonctions dans le sol et contribuent ainsi à la réalisation de services écosystémiques tels que la fertilité du sol, la résistance à l'érosion et la régulation du climat (Kibblewhite et al., 2008).

Les écosystèmes terrestres sont soumis à de nombreuses perturbations dues aux activités anthropiques. L'agriculture intensive est l'une des principales causes de perturbations des sols et constitue aujourd'hui une menace majeure envers la biodiversité (Robertson and Swinton, 2005; Tsiafouli et al., 2015; IPBES, 2019). Ainsi, la densité et la diversité des organismes du sol sont plus faibles dans les agroécosystèmes que dans les autres écosystèmes (Joimel et al., 2017). Dans les systèmes de culture intensifs, de nombreuses perturbations physiques et chimiques du sol impactent les organismes du sol. En effet, les pratiques agricoles telles que le travail du sol, les traitements phytosanitaires ou le type de fertilisation peuvent avoir des effets directs ou indirectes sur les organismes du sol (Kladivko, 2001; Cortet et al., 2002; Miyazawa et al., 2002).

Le développement de systèmes alternatifs basés sur de moindres perturbations physiques et chimiques du sol, tels que l'agriculture biologique et l'agriculture de conservation des sols, pourrait permettre de minimiser l'impact des systèmes de culture sur les organismes du sol et d'améliorer les services écosystémiques associés. Toutefois, la plupart des études sur le milieu agricole sont menées sur la biodiversité aérienne, tandis que l'effet des systèmes de culture alternatifs sur la biodiversité du sol reste relativement peu connu (Tableau 1). Le manque d'études empêche d'atteindre un consensus sur les systèmes qui permettraient de promouvoir la biodiversité des sols agricoles (Christel et al., 2021). De plus, l'effet des systèmes en transition vers ces alternatives sur la biodiversité des sols est encore mal connu, en particulier lors de la transition vers l'agriculture de conservation ou d'un système alternatif à un autre.

A l'heure actuelle, de nombreuses lacunes persistent dans nos connaissances sur l'effet des systèmes de culture sur la biodiversité des sols notamment sur : 1) la biodiversité des sols dans les systèmes de culture alternatifs (biologique, conservation des sols), tout particulièrement pour les systèmes en agriculture de conservation qui sont peu étudiés en Europe, 2) les changements qui surviennent dans les communautés d'organismes du sol durant la période de transition vers ces alternatives, ou d'une alternative à l'autre, et 3) l'évaluation simultanée de la densité et de la diversité de plusieurs groupes d'organismes (microorganismes, mésofaune, macrofaune) et des fonctions associées dans les sols agricoles.

La variabilité des résultats concernant la densité et la diversité des organismes du sol dans les systèmes alternatifs est large et reste encore mal comprise. Cette variabilité peut être en partie

due à la diversité des pratiques au sein même des systèmes. Le choix des méthodes pour caractériser d'une part la biodiversité et d'autre part les systèmes de culture joue un rôle important dans l'interprétation des résultats et la gestion de la variabilité.

Tableau 1. Synthèse sur les effets de l'agriculture biologique et de l'agriculture de conservation des sols sur l'abondance, la diversité et la structure des communautés des organismes du sol, d'après des exemples issus de la littérature. ↗ : augmentation, ↘ : diminution ou → : absence d'effet par rapport à l'agriculture conventionnelle.

	Agriculture biologique	Agriculture de conservation
Abondance & biomasse	↗: biomasse et activité microbiennes (Birkhofer et al., 2008; Lori et al., 2017), microarthropodes (Doles et al., 2001; Bettiol et al., 2002), macroarthropodes (Maeder et al., 2002; Birkhofer et al., 2008), vers de terre (Bettiol et al., 2002; Maeder et al., 2002; Pelosi et al., 2015) → or ↘: microorganismes (Bettiol et al., 2002; Schrader et al., 2006), microarthropodes (Schrader et al., 2006; Potapov et al., 2022), macroarthropodes (Patterson et al., 2019), vers de terre (Pelosi et al., 2009)	↗: biomasse microbienne (Sapkota et al., 2012; Palm et al., 2014), bactéries et champignons (Henneron et al., 2015), mésofaune (Ayuke et al., 2019; Dulaurent et al., 2022), macroarthropodes (Ayuke et al., 2019), vers de terre (Mele and Carter, 1999; Castellanos-Navarrete et al., 2012; Hernández et al., 2017; Dulaurent et al., 2022) → or ↘: biomasse microbienne (Scopel et al., 2013)
Diversité	↗: bactéries et champignons (Hartmann et al., 2015; Lupatini et al., 2017), microarthropodes (Bettiol et al., 2002; Schrader et al., 2006), macroarthropodes (Patterson et al., 2019), vers de terre (Henneron et al., 2015) → or ↘: microarthropodes (Alvarez et al., 2001; Doles et al., 2001; Filser et al., 2002; Peredo et al., 2009; Potapov et al., 2022), vers de terre (Pelosi et al., 2009)	↗: mésofaune and macrofaune (Ayuke et al., 2019), champignons (Choudhary et al., 2018), bactéries (Singh et al., 2020) → or ↘: vers de terre and collemboles (Dulaurent et al., 2022), champignons (Mhlanga et al., 2022; Zhang et al., 2022)
Structure des communautés	Microorganismes (Hartmann et al., 2015), vers de terre (Capowiez et al., 2009a), collemboles (Potapov et al., 2022)	Microorganismes (Romero-Salas et al., 2021; Zhang et al., 2022), vers de terre (Pelosi et al., 2009)

Objectif : L'objectif principal de cette thèse est de caractériser l'effet des systèmes de culture alternatifs stables ou en transition, en comparaison à l'agriculture conventionnelle, sur les organismes du sol et les services écosystémiques associés. Ce travail s'appuie sur l'évaluation de multiples organismes du sol (microorganismes, mésofaune, macrofaune), leurs interactions, et plusieurs fonctions associées (décomposition de la litière, minéralisation du carbone et de l'azote, stabilité structural du sol) qui sont impliquées dans la réalisation de services écosystémiques. Plus largement, ce travail vise à court terme à améliorer nos connaissances sur l'écologie des sols en milieu agricole, et à plus long terme à donner des clés pour le développement d'agroécosystèmes durables qui s'appuient sur une importante biodiversité et sur le fonctionnement des sols. Ce travail vise à répondre à plusieurs questions : 1) Quels sont les effets des systèmes de culture alternatifs sur la biodiversité des sols et les fonctions associées ? ; 2) Comment les organismes et les fonctions du sol répondent à la transition vers ces systèmes alternatifs ? ; 3) Quelles sont les pratiques agricoles qui ont l'impact le plus fort sur les organismes du sol ? ; et 4) Quelles approches permettent de caractériser au mieux les effets des systèmes de culture sur la biodiversité et le fonctionnement des sols ?

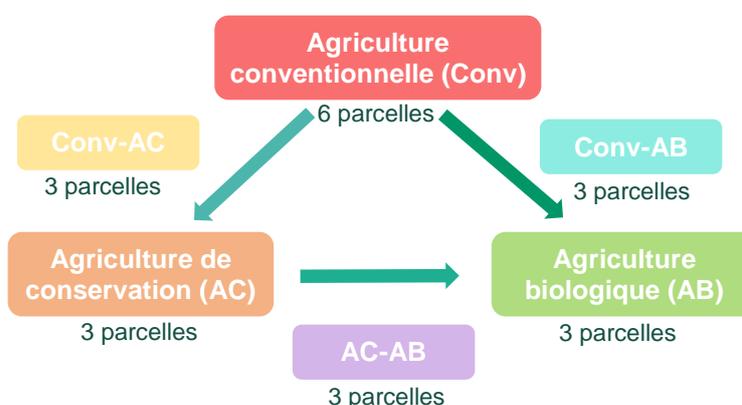
Méthode : Les résultats de la thèse ont été obtenus en combinant des mesures réalisées sur le terrain et en laboratoire. Des prélèvements de sol et d'organismes du sol ont été réalisés à l'automne 2020 puis 2021 sur 21 parcelles appartenant à des agriculteurs situés dans les régions Centre et Ile-de-France (France). L'objectif était d'observer les organismes du sol au début et un an après la culture du blé dans différents systèmes de culture.

Six systèmes de culture ont été étudiés : i) trois systèmes stables établis de sept à plus de vingt ans en agriculture conventionnelle (Conv, n = 6 parcelles), biologique (AB, n = 3) ou de conservation (AC, n = 3) et ii) trois systèmes en transition vers des systèmes alternatifs depuis deux à trois ans, de l'agriculture conventionnelle vers l'agriculture biologique (Conv-AB, n = 3) ou vers l'agriculture de conservation (Conv-AC, n = 3), ou de l'agriculture biologique vers l'agriculture de conservation (AC-AB, n = 3) (Tableau 2).

Tableau 2. Localisation, cultures et systèmes de culture pour les 21 parcelles de l'étude. L'âge correspond au nombre d'années depuis la conversion vers le système observé en 2020. Conv : agriculture conventionnelle, AC : agriculture de conservation, AB : agriculture biologique, Conv-AC, Conv-AB, AC-AB : transitions système initial-système final (≤ 3 ans).

Parcelle	Département	Culture 2019	Culture 2020	Culture 2021	Système	Age
A1	Eure-et-Loir	colza	blé	blé	Conv-AC	3
A2	Essonne	féverole	blé	couvert	AC	17
A3	Essonne	luzerne	blé	blé	AB	20
A4	Yvelines	triticale, pois	blé	sol nu	AB	19
A5	Eure-et-Loir	colza, lin, pois	blé	orge	Conv	20
A6	Essonne	lentille	blé	sol nu	AC-AB	2
A7	Yvelines	couvert	blé	seigle	Conv-AB	2
A8	Essonne	colza	blé	moutarde	AC	7
A9	Yvelines	féverole	blé	colza	Conv	20
A10	Eure-et-Loir	colza	blé	luzerne	Conv	20
A11	Yvelines	colza	blé	couvert	AB	20
A12	Yvelines	colza	blé	couvert	Conv	20
A13	Yvelines	maïs	blé	seigle, lentille	AC-AB	2
A14	Yvelines	orge	blé	couvert	Conv-AC	3
A15	Yvelines	féverole	blé	blé	Conv-AC	3
A16	Essonne	colza	blé	moutarde	Conv	20
A17	Yvelines	orge	blé	colza	AC	10
A18	Yvelines	luzerne, blé	blé	sol nu	Conv-AB	3
A19	Yvelines	raygrass	blé	trèfle	Conv-AB	2
A20	Eure-et-Loir	triticale, féverole	blé	couvert	AC-AB	2
A21	Eure-et-Loir	colza	blé	orge	Conv	20

Figure 1. Systèmes de culture stables et en transition étudiés lors de la thèse et nombre de parcelles échantillonnées pour chaque système. Les abréviations présentées sont utilisées dans la suite du résumé.



Des enquêtes ont été menées auprès de agriculteurs afin d’obtenir les informations concernant les pratiques appliquées sur les parcelles échantillonnées. A partir de ces données, des indicateurs ont été calculés pour évaluer le travail du sol, les traitements phytosanitaires, la fertilisation et la diversification des cultures. Ces indicateurs ont ensuite été combinés afin d’obtenir des indices composites représentant l’intensité du travail du sol (Itill), des traitements phytosanitaires (Itreat) et de la fertilisation (Ifertil) selon la méthodologie présentée par Büchi et al. (2019) (Tableau 3).

Tableau 3. Indicateurs primaires et indices composites (en gras) représentant l’intensité des pratiques dans les systèmes de culture. La colonne “durée” représente la durée sur laquelle les données sont collectées pour le calcul de l’indicateur. Les indicateurs et indices sont adaptés de l’étude de Büchi et al. (2019).

Catégories	Nom	Durée	Description
(a) Diversification des cultures	nbCrop	5 ans	Nombre de cultures différentes
(b) Perturbations physiques du sol (travail du sol)	nbDTill	1 an	Nombre de labours profonds (i.e. > 15 cm)
	nbSTill	1 an	Nombre de travaux superficiels
	soilP	5 ans	Type de travail du sol, moyenne du poids annuel du travail du sol (labour = 0.5, travail réduit = 3, non-labour = 5)
Itill Moyenne indicateurs normalisés : (nbDTill + nbSTill + (1 - soilP)) / 3			
(c) Protection phytosanitaire (pesticides)	nbHerb	1 an	Nombre de traitements herbicides
	nbFung	1 an	Nombre de traitements fongicides
	nbInsect	1 an	Nombre de traitements insecticides
	Itreat Nombre total de traitements : herbicides, fongicides, insecticides, molluscicides and régulateurs de croissance		
IFT Indice de fréquence des traitements			
(d) Fertilisation	nbOrg	5 ans	Nombre d’apports de fertilisants organiques
	qminN	1 an	Quantité d’apports des engrais minéraux azotés (kg N.ha ⁻¹)
	resExp	5 ans	Nombre d’exportation des résidus
	Ifertil Moyenne indicateurs normalisés : (nbOrg + qminN + resExp) / 3		

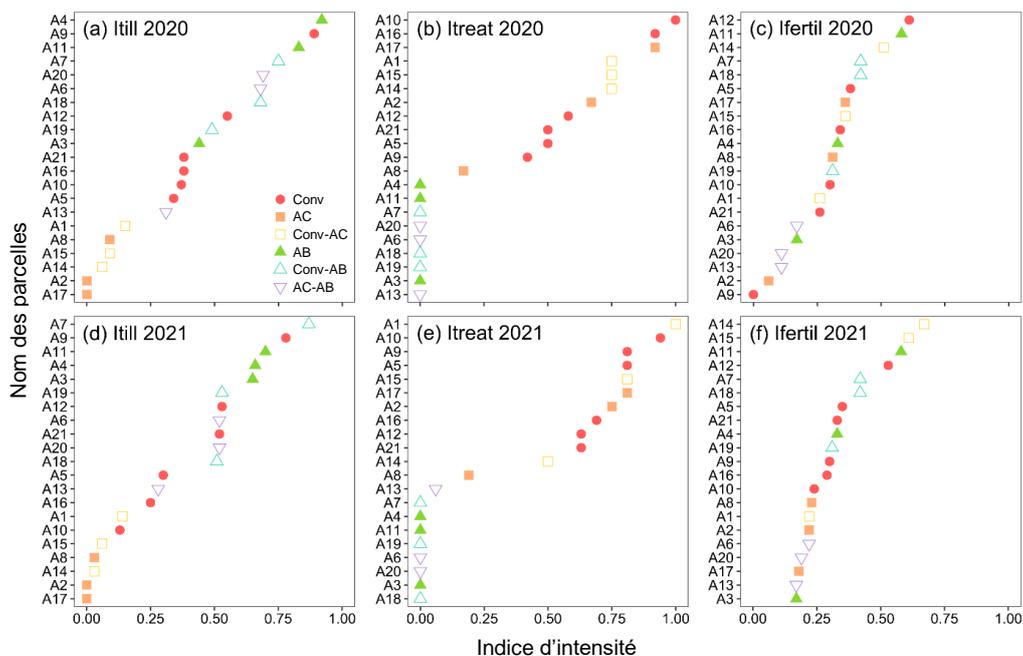


Figure 2. Indices composites de l’intensité du travail du sol (Itill), des traitements phytosanitaires (Itreat) et de la fertilisation (Ifertil) sur les parcelles étudiées. Les symboles représentent les systèmes de culture auxquels appartiennent les parcelles (cf. légende). Conv : agriculture conventionnelle, AC : agriculture de conservation, AB : agriculture biologique, Conv-AC, Conv-AB, AC-AB : transitions système initial-système final (≤ 3 ans).

Des échantillons de sol ont été prélevés en 3 points de la parcelle (i.e. répliquats) (Figure 2) afin d'analyser les propriétés du sol, les organismes du sol (microorganismes, mésofaune, macrofaune), la stabilité de la structure et les cinétiques de minéralisation C et N. De plus, des sachets de litière ont été mis en place sur les parcelles pendant 5 mois afin de mesurer la décomposition de la litière in situ.

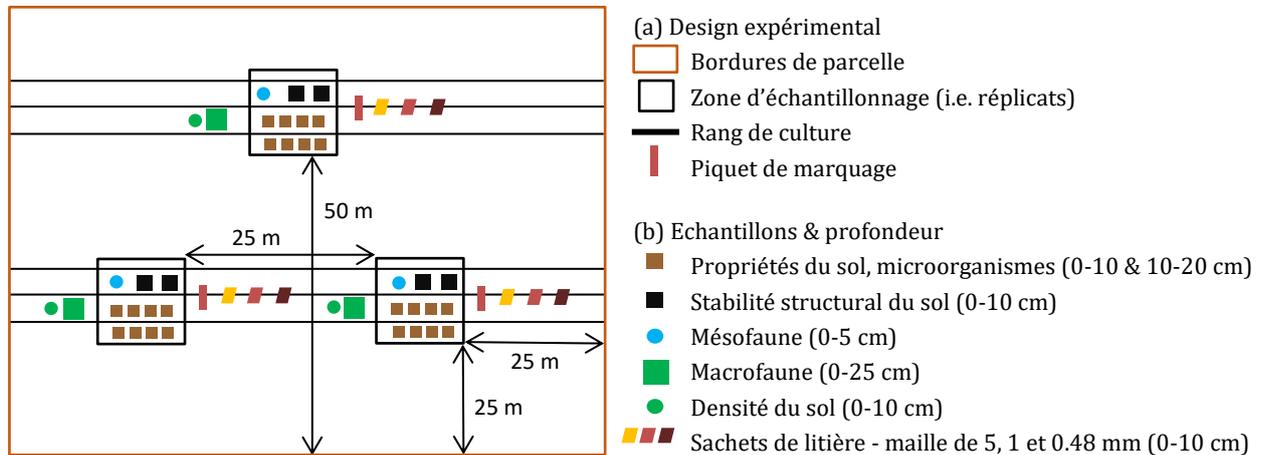


Figure 3. Représentation schématique du design expérimental et des échantillons prélevés lors de la thèse.

Chapitre 1 – Utilisation des indicateurs d'intensité des pratiques pour révéler l'effet des systèmes de culture sur la mésofaune du sol

Le développement de systèmes de culture qui reposent sur de moindres perturbations du sol et de la biodiversité requiert de comprendre les conséquences des différentes pratiques agricoles sur les organismes du sol. Toutefois, la plupart des études caractérisent les systèmes de culture en distinguant les principaux types de systèmes (ex. conventionnel, biologique, conservation des sols) sans prendre en compte la diversité des pratiques au sein de ces systèmes. L'objectif de ce chapitre est de décrire de façon plus précise les systèmes de culture et leurs effets sur la mésofaune du sol à l'aide d'indicateurs d'intensité des pratiques développés par des agronomes.

La mésofaune du sol a été échantillonnée au moyen de cylindres de sol de 5 cm de diamètre sur les 21 parcelles étudiées, à l'automne 2020 et 2021. La densité de la mésofaune du sol et la diversité taxonomique et fonctionnelle des collemboles ont été déterminés et mises en relation avec les systèmes de culture et l'intensité des pratiques. La diversité fonctionnelle des collemboles a été évaluée en utilisant les traits fonctionnels des espèces issus d'une base de données (BETSI) et en réalisant des mesures directement sur les individus collectés (longueur du corps, de la tête, des pattes, des antennes et de la furca).

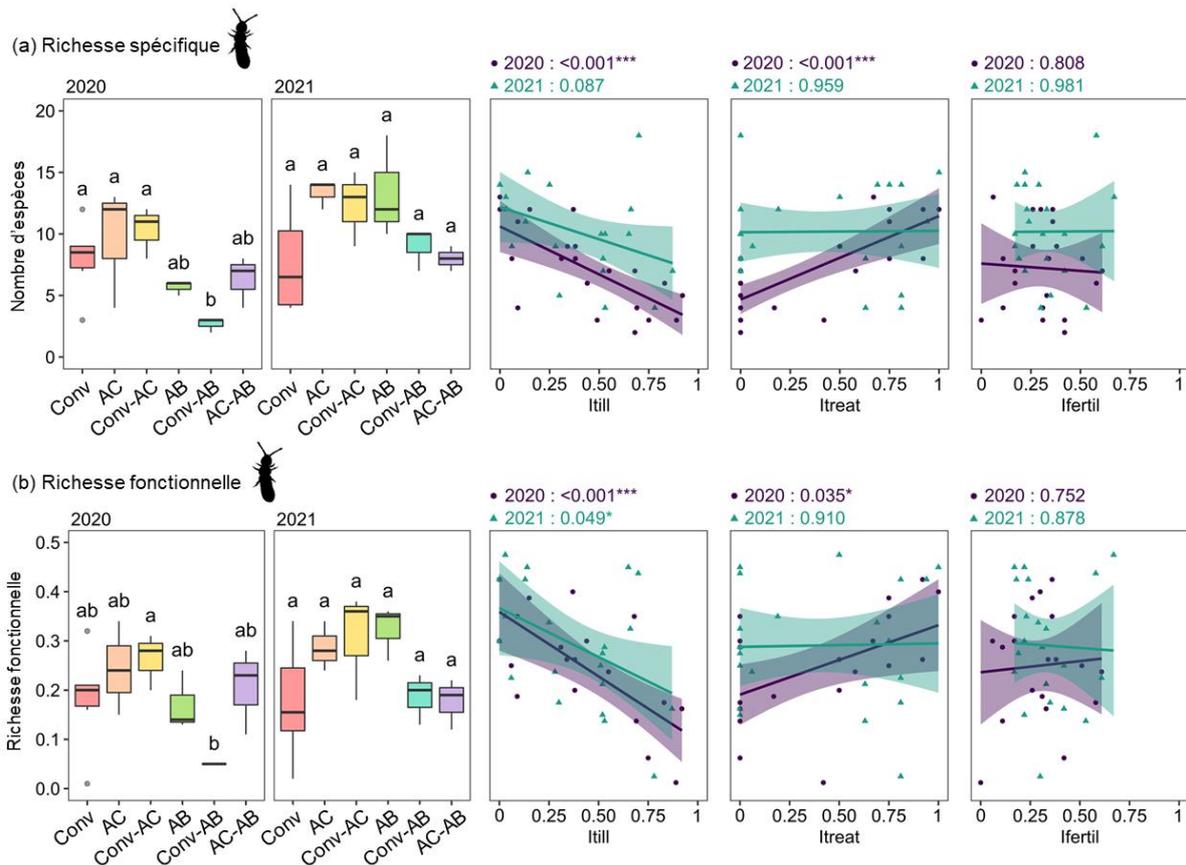


Figure 4. Effet des systèmes de culture et de l'intensité des pratiques sur (a) le nombre d'espèce et (b) la richesse fonctionnelle des collemboles en 2020 et 2021. Les différences significatives entre systèmes sont représentées avec des lettres différentes et les effets significatifs des indices d'intensité sont reportés tels que : $P < 0.05$ *, < 0.01 **, < 0.001 ***. Conv : agriculture conventionnelle, AC : agriculture de conservation, AB : agriculture biologique, Conv-AC, Conv-AB, AC-AB : transitions système initial-système final (≤ 3 ans).

Nos résultats montrent que les paramètres de la mésofaune du sol varient entre les systèmes de culture, mais que la variabilité entre les années et les parcelles est forte (Figure 3). La densité de la mésofaune tend à être plus faible dans les systèmes biologiques que conventionnels et de conservation en 2020, et plus forte dans les systèmes de conservation, particulièrement pour les collemboles, en 2021. Les systèmes en transition vers l'agriculture biologique présentent une faible densité de la mésofaune. La densité de la mésofaune et la diversité des collemboles diminuent lorsque l'intensité du travail du sol augmente les deux années, et lorsque que l'intensité des traitements phytosanitaires diminue en 2020 uniquement. Les indicateurs d'intensité des pratiques révèlent un effet variable de la fertilisation, avec l'absence d'effet de l'intensité totale de la fertilisation, mais un effet négatif du nombre d'apports de fertilisants organiques notamment.

L'utilisation des traits fonctionnels permet de mettre en évidence des effets de l'intensité des pratiques sur les collemboles qui ne sont pas observés avec la diversité des espèces. En particulier, les traits correspondant à des espèces adaptées à la surface du sol (ex. trichobotries, ocelles) sont moins représentés à mesure que l'intensité du travail du sol augmente. De plus, les traits mesurés permettent d'observer que la longueur du corps et des appendices des collemboles tendent à être

plus grands lorsque l'intensité du travail du sol est faible et l'intensité des traitements phytosanitaires est élevée. Les différents traits mesurés semblent toutefois suivre la même tendance. La mesure de la longueur du corps pourrait donc être suffisante pour mieux caractériser l'effet des agrosystèmes sur les collemboles.

Plus généralement, les résultats obtenus indiquent que : 1) les indicateurs d'intensité des pratiques peuvent permettre une meilleure compréhension des effets des systèmes de culture sur la mésofaune du sol, 2) les approches taxonomique et fonctionnelle de la diversité sont complémentaires pour évaluer l'effet des pratiques sur les collemboles, et 3) les traits fonctionnels issus de base de données et la mesure de la longueur du corps permettent de révéler l'effet des perturbations sur les communautés de collemboles en milieu agricole. Cependant, la variabilité des résultats reste importante, et de nouvelles études pourraient permettre d'évaluer pleinement la pertinence des indicateurs d'intensité des pratiques et des traits fonctionnels pour caractériser l'effet des systèmes de culture sur la biodiversité du sol.

Chapitre 2 – L'intensité des pratiques dans les systèmes de culture influence des groupes spécifiques au sein de la macrofaune du sol

D'après de précédentes études, les organismes du sol de plus grande taille pourraient être les plus sensibles aux perturbations liées aux pratiques agricoles dans les systèmes de culture, notamment concernant le travail du sol. La macrofaune du sol comprend des groupes d'organismes qui présentent des caractéristiques morphologiques et écologiques variées, lesquelles impliquent des réponses variées de ces groupes aux pratiques agricoles. Dans ce chapitre, l'objectif est d'évaluer les effets des systèmes de culture et de l'intensité des pratiques sur la macrofaune du sol, et en particulier sur les macroarthropodes et les vers de terre.

La macrofaune du sol a été échantillonnée en réalisant un tri manuel de blocs de sol de 25 x 25 x 25 cm de côté sur les 21 parcelles étudiées, à l'automne 2020 et 2021. La densité des différents groupes de macrofaune ainsi que la biomasse, la diversité taxonomique et fonctionnelle des vers de terre ont été évaluées.

La densité et de diversité de la macrofaune (macroarthropodes et vers de terre) montrent relativement peu de différences entre les différents systèmes de cultures et répondent peu à l'intensité des pratiques. Les effets observés varient entre les groupes d'organismes et les années. Certains groupes de macroarthropodes répondent à l'intensité du travail du sol et à la fertilisation (chilopodes, coléoptères, diplopodes) et aux traitements phytosanitaires (araignées, chilopodes, diplopodes), mais ces effets ne sont pas significatifs pour les deux années. De plus, la densité et la biomasse des vers de terre épi-anéciques juvéniles et des espèces de petite taille diminuent sur les parcelles où l'intensité du travail du sol est plus élevée. Une augmentation de l'intensité de la

fertilisation a un effet négatif sur la densité et la biomasse des vers de terre épi-anéciques, et inversement a un effet positif sur les vers de terre endogés adultes, et pourrait être liée à l'occurrence de plusieurs traits fonctionnels des vers de terre (ex. taille du corps, ratio masse/longueur, type d'épithélium). Toutefois, les approches taxonomiques et fonctionnelles de la diversité de vers de terre conduisent à des conclusions similaires en raison du faible nombre d'espèces.

Ces résultats soutiennent le besoin de prendre en compte l'intensité des pratiques agricoles ainsi que d'évaluer différents groupes taxonomiques, trophiques et écologiques afin d'évaluer l'effet des systèmes de culture sur la macrofaune du sol.

Chapitre 3 – Les systèmes de culture et l'intensité des pratiques affectent les communautés microbiennes et les traits bactériens dans les sols agricoles

Les microorganismes sont essentiels au fonctionnement des sols agricoles et peuvent bénéficier d'une gestion moins intensive des systèmes de culture. La compréhension de l'effet des systèmes de culture alternatifs sur les communautés microbiennes du sol reste limitée, en particulier en ce qui concerne l'agriculture de conservation (Christel et al., 2021). L'objectif de ce chapitre est d'étudier l'effet des différents systèmes de culture (conventionnel, biologique, de conservation ; stable ou en transition) et de l'intensité des pratiques (travail du sol, traitements phytosanitaires, fertilisation) sur l'abondance, la structure des communautés et la diversité bactériennes et fongiques du sol.

Des échantillons de sol ont été prélevés à 0-10 et 10-20 cm de profondeur sur les 21 parcelles étudiées, à l'automne 2020 et 2021. Ces échantillons ont été utilisés pour la caractérisation des propriétés du sol, de la biomasse microbienne moléculaire et des communautés bactériennes et fongiques du phylum à l'espèce grâce à un séquençage à haut débit. De plus, les traits fonctionnels bactériens ont été évalués à partir des unités taxonomiques identifiées et de la base de données BactoTraits (Cébron et al., 2021) utilisée ici pour la première fois en milieu agricole.

L'abondance fongique, l'abondance bactérienne et la diversité fongique sont plus élevées dans les systèmes en transition vers l'agriculture biologique (Conv-AB) et plus faibles dans les systèmes de conservation stables (AC). Nous n'avons pas observé d'effets significatifs des systèmes sur la biomasse microbienne et l'abondance bactérienne. Toutefois, les diversités bactérienne et fongique sont significativement plus élevées (indices de Shannon et d'équitabilité) lorsque l'intensité du labour augmente. De plus, la diversité fongique est plus élevée lorsque l'intensité des traitements phytosanitaires diminue. La composition des communautés microbiennes semble être influencée davantage par les propriétés du sol, même si celles-ci varient

relativement peu entre les parcelles, que par les systèmes. Cependant, les traits bactériens révèlent des différences importantes entre les communautés bactériennes des systèmes Conv-AC et des systèmes Conv et AC. Plus précisément, de nombreux traits bactériens sont influencés par l'intensité des pratiques agricoles. Sur les deux années, plusieurs traits observés à 10-20 cm de profondeur sont influencés par l'intensité du travail du sol (ex. pH et température de croissance, forme, type trophique), tandis que l'intensité des traitements phytosanitaires influence majoritairement la mobilité des bactéries à 0-10 cm de profondeur.

Ainsi, nos résultats suggèrent que les propriétés des sols ainsi que les systèmes de culture et l'intensité du travail du sol sont des facteurs qui influencent fortement les communautés bactériennes et fongiques dans les sols agricoles. De plus, les traits bactériens sont des indicateurs importants pour comprendre l'effet des systèmes de culture sur les communautés microbiennes.

Chapitre 4 – Vers une évaluation de plusieurs groupes d'organismes du sol et de leurs interactions dans des systèmes de culture alternatifs et en transition

Parmi les études menées sur la biodiversité du sol dans les systèmes de culture, peu s'intéressent simultanément à différents groupes d'organismes du sol et à leurs interactions. Les objectifs de ce chapitre sont : 1) de synthétiser l'effet des systèmes en agriculture biologique et de conservation sur la densité des différents groupes d'organismes, en comparaison à l'agriculture conventionnelle, et 2) d'observer l'impact des pratiques agricoles sur les groupes trophiques et plus largement sur le réseau trophique du sol.

Tout d'abord, nous avons synthétisé l'effet des systèmes alternatifs, stables ou en transition, sur les microorganismes, la mésofaune et la macrofaune du sol. Des « effets de taille » (*effect size*) ont été calculés pour estimer les différences de densité entre les systèmes alternatifs et conventionnels d'une part, et les systèmes en transition et stables (équivalent des systèmes en place avant la transition) d'autre part.

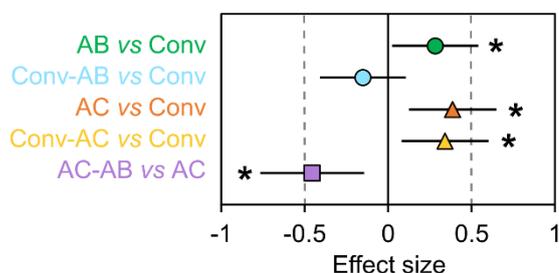


Figure 5. Effet de taille moyen (Hedge's estimate, intervalle de confiance à 95%) des systèmes alternatifs stables ou en transition sur la densité des organismes du sol. Inclut les données 2020 et 2021. L'effet de taille est significativement positif ou négatif (*) lorsque que l'intervalle de confiance n'inclut pas le zéro.

Nos résultats montrent que les systèmes AB et AC ont un effet moyen positif sur la densité des organismes du sol par rapport aux systèmes Conv. Les systèmes en transition Conv-AC suivent la même tendance, tandis que les systèmes en transition AC-AB ont un effet moyen négatif sur les organismes du sol en comparaison aux systèmes AC. Plus précisément, nous avons observé que : 1) les systèmes AC ont un effet positif sur la densité des collemboles, larves de Coléoptères et gastropodes (i.e. limaces) en comparaison aux systèmes Conv, 2) les systèmes AB n'ont pas d'effet significatif mais tendent à favoriser la densité des microorganismes et de certains groupes de macrofaune (ex. diplopodes) en comparaison aux systèmes Conv, et 3) les systèmes en transition Conv-AB et Conv-AC ont un effet positif sur la densité de vers de terre.

Dans un second temps, nous avons estimé l'effet de l'intensité des pratiques sur les interactions entre les groupes trophiques du sol et plus largement sur le réseau trophique du sol. Pour cela, nous avons conduit une modélisation par équations structurales (*Structural equation modeling* ou *SEM*) visant à évaluer les relations entre l'intensité des pratiques (travail du sol, traitements phytosanitaires, apports de matière organique), la matière organique du sol et les groupes trophiques du sol.

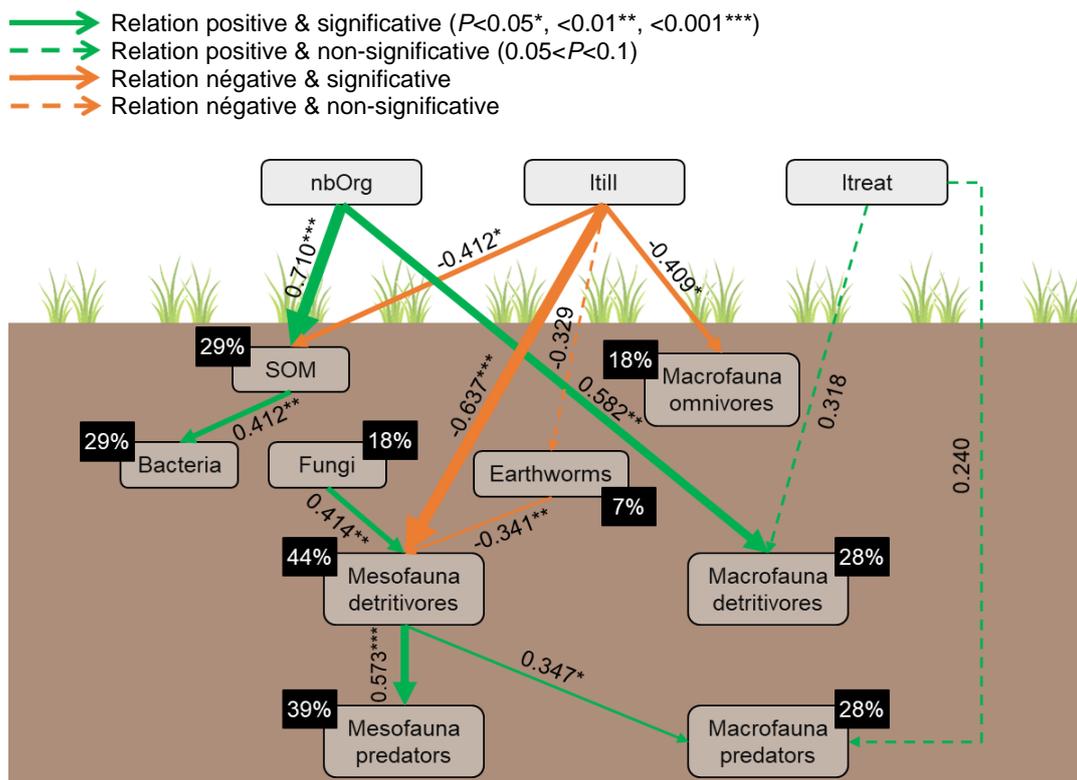


Figure 6. Modèle représentant les relations entre l'intensité du travail du sol (Itill), les traitements phytosanitaires (Itreat), le nombre d'apport de matière organique (nbOrg), le contenu du sol en matière organique (SOM) et la densité des groupes trophiques du sol (bactéries, champignons, mésofaune détritivore et prédatrice, macrofaune omnivore, détritivore et prédatrice, vers de terre).

Le modèle final permet d'observer que le travail du sol a un fort effet négatif sur l'ensemble du réseau trophique du sol avec de nombreux effets directs ou indirects sur les différents groupes trophiques. A l'inverse, les apports de matière organique favorisent la densité des bactéries et la macrofaune détritivore (ex. diplopodes). De plus, nous avons observé que la mésofaune détritivore pourrait avoir une place centrale dans le réseau trophique des sols agricoles.

Pour synthétiser, notre étude démontre que les systèmes en agriculture de conservation et une faible intensité du travail du sol favorisent la densité de nombreux groupes d'organismes du sol et leurs interactions au sein du réseau trophique du sol. L'agriculture biologique et les apports de matière organique associés à ce système ont un effet moins marqué mais sur d'autres groupes d'organismes du sol que ceux favorisés par l'agriculture de conservation.

Chapitre 5 – Rôle des organismes de différentes classes de taille dans les sols cultivés : quels sont les apports des expériences avec des sachets de litière ? Une méta-analyse.

Ce chapitre correspond à un article publié dans la revue *Soil Biology and Biochemistry* (Chassain et al., 2021). Il contient une méta-analyse basée sur des données issues de la littérature scientifique. L'objectif de cette méta-analyse est d'estimer l'effet de la mésofaune et de la macrofaune du sol sur la décomposition de la litière en milieu agricole grâce à des expériences utilisant des sachets de litière avec plusieurs tailles de maille. De plus, la revue de la littérature a permis de dégager des recommandations pour le développement d'un protocole standardisé sur l'utilisation des sachets de litière en milieu agricole.

Les résultats de la méta-analyse indiquent que la macrofaune, la mésofaune et la combinaison de ces deux groupes augmentent significativement la décomposition de la litière. De plus, la mésofaune et la macrofaune contribuent équitablement à la décomposition et leurs effets ne sont pas additifs. Cependant, ces effets sont influencés par de nombreux paramètres tels que le climat, le type de sol, la culture et le système de culture en place.

Ainsi, les expériences de sachets de litières permettent de mettre en évidence que même dans les sols fortement perturbés, les organismes du sol de différentes classes de taille participent à la décomposition de la litière. De nombreuses lacunes persistent toutefois dans la littérature, notamment sur l'impact des systèmes alternatifs et des pratiques sur la décomposition.

Chapitre 6 – Les fonctions associées aux organismes du sol dans les systèmes alternatifs

Les services écosystémiques associés aux sols agricoles reposent fortement sur la relation entre les organismes du sol et les fonctions du sol, sous l'influence des pratiques agricoles et des

propriétés du sol. Cependant, les effets des systèmes de culture et de l'intensité des pratiques sur les fonctions du sol associées aux organismes du sol restent peu connus. Dans ce chapitre, nous avons mesuré la réalisation de plusieurs fonctions du sol dans les différents systèmes de culture étudiés, et discuté de leurs relations avec les organismes du sol et l'intensité des pratiques.

Des prélèvements de sol ont été réalisés à 0-10 and 10-20 cm de profondeur afin d'estimer le carbone organique du sol (SOC), l'azote total du sol (TN) et le stock de carbone du sol. La perte de masse de litière et l'effet de la méso- et de la macrofaune sur la décomposition de la litière ont été mesurées à l'aide de sachets de litière de trois tailles de maille (5 mm, 1 mm, 48 μ m) enterrés sur les parcelles pendant 5 mois. Les taux de minéralisation C et N ont été mesurés pendant 120 jours en réalisant des incubations de sols collectés à 0-10 cm de profondeur pour tous les systèmes, et également à 10-20 cm de profondeur pour les systèmes AC et Conv-AC. La stabilité des agrégats de sol à 0-10 cm de profondeur a été estimée en utilisant la méthodologie de Le Bissonnais (1996) et en déterminant le diamètre moyen pondéré des agrégats.

Les sols des systèmes AC et AB tendent à contenir davantage de SOC, à 0-10 et 10-20 cm de profondeur respectivement, que les sols des autres systèmes. Cependant, nous n'avons observé aucun effet significatif des systèmes de culture que ce soit sur la minéralisation C & N, sur le stock de COS à 0-20 cm de profondeur, ou sur la décomposition de la litière. La décomposition de la litière est plus élevée dans les sachets de maille 48 μ m lorsque l'intensité du travail du sol augmente et l'intensité des traitements phytosanitaires diminue, et dans les sachets de maille 1 mm lorsque que l'intensité de la fertilisation diminue. L'effet de la macrofaune sur la décomposition est significativement plus élevé dans les systèmes Conv-AB et AC-AB et diminue lorsque l'intensité des traitements phytosanitaires augmente. La minéralisation N (par kg sol et par g TN) tend à être plus élevée dans les systèmes AC et plus faible dans les systèmes AB à 0-10 cm de profondeur. Le C minéralisé après 120 jours (par kg sol) et le taux de minéralisation de la fraction lente du C (par kg sol et par g SOC) sont négativement impactées par le travail du sol et par l'intensité des traitements phytosanitaires. Le N minéralisé après 120 jours (par kg sol et par g TN) est négativement impacté par le travail du sol. De plus, la stabilité des agrégats du sol est significativement plus importante dans les systèmes AC que dans les systèmes Conv et AB, en lien avec une faible intensité du travail du sol.

Au final, les systèmes AC et AB influencent plusieurs fonctions du sol et plus largement les services écosystémiques associés. D'une part, l'AC favorise la stabilité des agrégats de sol et tend à accélérer la minéralisation N, ce qui suggère une plus faible sensibilité des sols à l'érosion et une augmentation des nutriments disponibles pour les cultures. Cependant, cela peut également conduire à une augmentation du risque de lessivage des nitrates et d'émissions azotées. A cela s'ajoute une pression sur les cultures due à la plus grande occurrence de limaces en AC. D'autre part, l'AB présente moins et de plus faibles effets sur les fonctions du sol, mais tend à augmenter

la décomposition de la litière (effet de la macrofaune et des microorganismes) et à ralentir la minéralisation C et N. Ces effets pourraient conduire à une potentielle augmentation du stockage du C dans les sols et participer ainsi à la régulation du climat, mais également à une plus faible disponibilité des nutriments pour les cultures et une potentielle baisse de rendement.

Nos résultats tendent à montrer des différences dans le fonctionnement des sols entre les systèmes alternatifs stables et les systèmes conventionnels, mais peu de différences lorsque les systèmes considérés sont en transition.

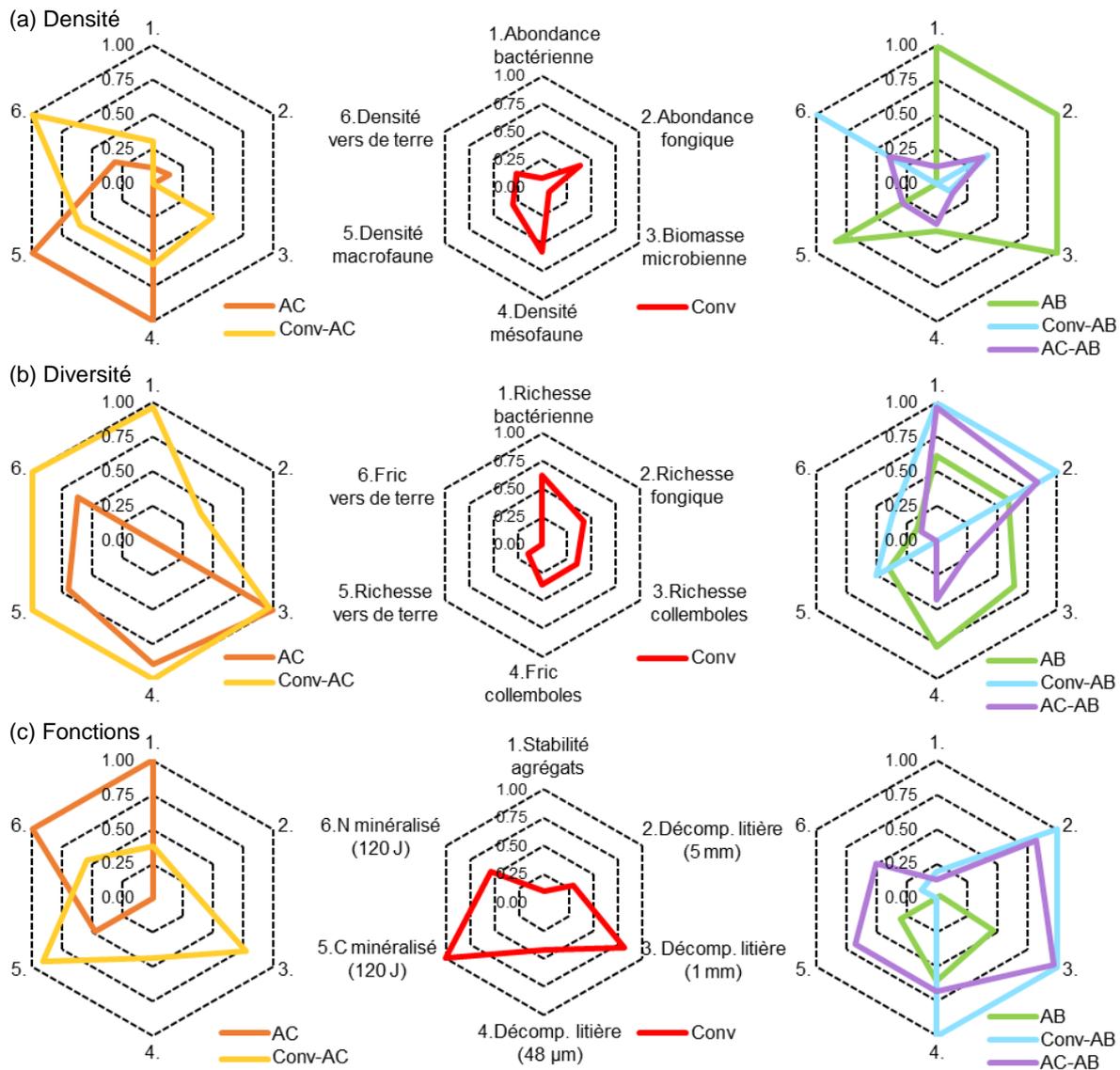
En conclusion

Ce travail de thèse présente plusieurs originalités. Les systèmes alternatifs habituellement étudiés indépendamment sont ici comparés dans une même étude, entre eux et par rapport aux systèmes conventionnels, à l'état stable et lors de la transition. De plus, l'évaluation menée sur la biodiversité des sols porte ici sur la densité et la diversité de plusieurs groupes d'organismes du sol, qui sont rarement étudiés simultanément, et sur leurs interactions au sein du réseau trophique du sol. La caractérisation de la biodiversité est associée à la mesure de plusieurs fonctions du sol liées aux activités des organismes, fournissant ainsi des informations sur les services écosystémiques maintenus par le sol dans les différents systèmes de culture. Enfin, ce travail repose sur plusieurs points de réflexion méthodologique : 1) l'utilisation des indicateurs d'intensité des pratiques, généralement utilisés par les agronomes, afin de mieux comprendre les perturbations causées par les systèmes de culture et leurs effets sur la biodiversité du sol, et 2) l'utilisation des traits fonctionnels pour plusieurs groupes d'organismes (collembolles, vers de terre, bactéries) afin d'obtenir une vision plus détaillée de la diversité des organismes du sol.

Nos résultats mettent en évidence le potentiel des systèmes alternatifs, reposant sur de moindres perturbations physiques et chimiques du sol, à promouvoir la densité et la diversité de la macrofaune, de la mésofaune et des microorganismes du sol, les interactions entre ces organismes au sein du réseau trophique du sol, et les fonctions associées dans les sols agricoles. Cependant, l'agriculture biologique et l'agriculture de conservation favorisent différents groupes d'organismes et différentes fonctions, et pourraient plus largement contribuer à favoriser différents services écosystémiques (Figure 7). L'agriculture de conservation a des bénéfices importants sur la biodiversité des sols et les fonctions associées en relation directe avec l'absence de travail du sol. L'agriculture biologique favorise quant à elle la biodiversité du sol grâce à l'absence de traitements phytosanitaires et aux apports plus importants de matière organique, mais via des effets plus faibles et plus variés.

De plus, les systèmes en transition présentent des effets spécifiques sur certains groupes d'organismes et certaines fonctions. Cela souligne le besoin d'études sur les systèmes lors des

premières années de la transition, en particulier pour les transitions vers ou depuis l'agriculture de conservation, et sur le long terme afin d'évaluer le temps nécessaire pour d'atteindre des caractéristiques similaires aux systèmes stables. Si toutes les pratiques peuvent influencer la biodiversité et les fonctions du sol, le travail du sol semble être le principal facteur d'influence de la biodiversité du sol en milieu agricole. Les effets des traitements phytosanitaires et de la fertilisation sont plus variables et difficiles à décoller de l'effet du travail du sol. De plus, la mésofaune est le groupe le plus impacté par l'intensité des pratiques agricoles, en particulier les détritivores qui semblent occuper une place centrale dans le réseau trophique des sols agricoles.



La mise en place de systèmes de culture alternatifs repose toutefois sur de nombreux facteurs. Notamment, le choix d'un système de culture dépend du contexte environnemental et vise à répondre aux risques environnementaux au niveau local. De plus, le développement et le choix de systèmes alternatifs doivent nécessairement prendre en compte le compromis entre la promotion de la biodiversité et des bénéfices environnementaux et la production afin de garantir la durabilité de ces systèmes.

Plus généralement, nos résultats illustrent l'absence d'une solution unique pour augmenter la durabilité des systèmes de culture. Il semble important de préciser qu'aucun des systèmes alternatifs ne doit pas être considéré comme la « meilleure » solution, mais que la coexistence de plusieurs alternatives pourrait permettre de tirer parti des avantages des différents systèmes et de limiter les menaces qui pèsent sur la biodiversité. Autrement dit, la promotion de la biodiversité du sol et la durabilité des systèmes de culture pourraient passer par la promotion d'une diversité de systèmes.

Au final, ce travail de thèse souligne le besoin de prise en compte de la biodiversité et du fonctionnement des sols lors de l'évaluation de la durabilité des systèmes de culture. Il est toutefois primordial de combiner les observations réalisées sur les compartiments aériens et souterrains de la biodiversité afin d'obtenir une vision globale de l'effet des systèmes de culture sur la biodiversité et les services écosystémiques.